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EVALUATION OF SEVERAL BIOLOGICAL MONITORING TECHNIQUES FOR HAZARD ASSESSMENT OF POTENTIALLY CONTAMINATED WASTEWATER AND GROUNDWATER

VOLUME 1 - ABERDEEN PROVING GROUND-EDGEWOOD AREA WASTEWATER TREATMENT PLANT

FINAL REPORT

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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

An evaluation of several biological monitoring techniques for hazard assessment of potentially contaminated effluent was conducted at the Aberdeen Proving Ground-Edgewood Area Wastewater Treatment Plant (APG-EA WWTP), Aberdeen Proving Ground, MD, from January 1989 to December 13, 1989. An array of biomonitoring tests structured in a tiered hazard assessment framework was used in the evaluation of the effluent. Several levels of biological organization were included in the array of tests.

Acute toxicity was evaluated on 24-h composite samples using a 15-min Microtox[R] assay which employs microbial (Photobacterium phosphoreum) bioluminescent activity. Two 24-h LC50 rotifer (Brachionus rubens) toxicity tests were conducted using 24-h composite samples. The following chronic tests were all performed two times using 24-h composite samples: 96-h EC50 algal (Selenastrum capricornutum) growth test, 7-d daphnid (Ceriodaphnia dubia) survival and reproduction test, and 7-d fathead minnow (Pimephales promelas) survival and

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phosphoreum, Rotifer, *Brachionus rubens*, green alga, *Selenastrum capricornutum*, daphnid, *Ceriodaphnia dubia*, fathead minnow, *Pimephales promelas*, African clawed frog, *Xenopus laevis*, Japanese medaka, *Oryzias latipes*, bluegill, *Lepomis macrochirus*.

19. ABSTRACT (Continued)

growth test. Generally, the acute rotifer tests and all chronic tests were conducted during the same periods in order to compare toxicological responses between biomonitoring systems.

Mutagenicity assays (Ames) were performed six times on the effluent and twice on the diluent water using 24-h composite samples. One preliminary 96-h (flow-through) teratogenicity test was conducted using the African clawed frog (*Xenopus laevis*) embryo teratogenesis assay (FETAX). A preliminary 6-month carcinogenicity test was conducted under flow-through test conditions with Japanese medaka (*Oryzias latipes*) unexposed fry and fry initiated with diethylnitrosoamine. The U. S. Army Biomedical Research and Development Laboratory's (USABRDL) 21-d bluegill (*Lepomis macrochirus*) computerized ventilatory monitoring system, which has been designed to detect unexpected abrupt changes in water quality or episodic events, was tested four times. Comprehensive chemical analyses were performed seven times on 24-h composite samples of both the effluent and diluent water. Routine water quality was also determined frequently throughout the 6-month carcinogenicity study.

EXECUTIVE SUMMARY

An evaluation of several biological monitoring techniques for hazard assessment of potentially contaminated effluent was conducted at the Aberdeen Proving Ground-Edgewood Area Wastewater Treatment Plant (APG-EA WWTP), Aberdeen Proving Ground, MD, from January 1989 to December 13, 1989. An array of biomonitoring tests structured in a tiered hazard assessment framework was used in the evaluation of the effluent. Several levels of biological organization were included in the array of tests.

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The array of biological monitoring techniques used to assess the potential toxicity of the APG-EA WWTP effluent showed that the effluent generally was not toxic during most of the study period. Acute toxicity was found in ≈3% of the effluent samples measured (toxicity occurred 3 days out of 95 days of sampling) via Microtox®. Three 15-min EC50s, which ranged from 1.1 to 18.8% effluent by volume, occurred during the period July 20-26, 1989. No acute toxicity was found in the 24-h rotifer tests.

No significant chronic toxicity was detected by the three biomonitoring systems used during two separate sets of tests. The effluent was not toxic to the green alga or the daphnid. A statistically significant (alpha = 0.05) reduction in fathead minnow larval growth occurred in the 50% effluent by volume treatment only during the second test. However, it is not clear why a reduction in growth occurred in the 50% effluent by volume treatment when a reduction did not occur in the 100% effluent by volume treatment. The reduction in growth at 50% effluent by volume may be attributable to statistical chance, i.e., 1 in 20 times one can expect a random event to occur.

No mutagenicity was detected in unconcentrated APG-EA WWTP effluent or unconcentrated dechlorinated APG diluent water. All concentrated (10X) effluent samples had mutagenic activity. No teratogenicity data are available because the test was a preliminary study. Likewise, no data are available from Carcinogenicity Test (O) because the test was a preliminary study.

No abrupt changes in effluent quality or episodic events were detected during Ventilatory Tests I, II, or IV. The effluent was not overtly toxic to the bluegills during the 14-d definitive phases of each test. That is, significant mortality did not occur during 14 d of exposure to 100% effluent. A number of plant operation problems occurred during Ventilatory Test III which ultimately caused the test to be terminated before it was completed. During Ventilatory Test III, the system detected both an increase in sediment load and chlorine concentration during separate episodes.

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TABLE OF CONTENTS

LIST OF TABLES

SECTION 1

INTRODUCTION

The Johns Hopkins University/Applied Physics Laboratory-Aquatic Ecology Section (JHU/APL-AES) under contract to the Health Effects Research Division of the United States Army Biomedical Research and Development Laboratory (USABRDL) conducted an on-site study from January 1989 to December 13, 1989 to determine the use of several biological monitoring techniques for hazard assessment of potentially contaminated effluent at the Aberdeen Proving Ground-Edgewood Area Wastewater Treatment Plant (APG-EA WWTP), Aberdeen Proving Ground, MD. The first five months of the study were used for facility modification, set-up, and range finding tests. The definitive experimental phase of the study was conducted over a 6-month period from June 14, 1989 to December 13, 1989.

APG-EA WWTP effluent (NPDES Permit No. MD 0021229; Outfall 001) used in the study was the final tertiary treated product of a raw influent which included a variable combination of domestic, munitions, and industrial sources (Nemeth, 1989). The plant has a designed capacity of 2.8 mgd; however, the actual capacity was 2.0 mgd with an average of 0.8 mgd (Logan, 1992). Chlorination was used for disinfection followed by dechlorination (sulfur dioxide) of the effluent before discharge.

An array of biomonitoring tests structured in a tiered hazard assessment framework was used in the evaluation of the effluent. Several levels of biological organization were included in the array of tests. The effluent was tested for acute and chronic toxicity; mutagenic, teratogenic, and carcinogenic potential; and chemical composition. In addition, USABRDL's biological monitoring early warning system was tested.

SECTION 2

OBJECTIVES OF STUDY

1) To evaluate acute toxicity of the effluent using the 15-min Microtox® procedure (Photobacterium phosphoreum bioluminescent activity) and the 24-h LC50 Rotifer Toxkit™ (Brachionus rubens) screening test.

2) To evaluate chronic toxicity using the 96-h EC50 algal (Selenastrum capricornutum) growth test, 7-d daphnid (Ceriodaphnia dubia) survival and reproduction test, and 7-d fathead minnow (Pimephales promelas) survival and growth test.

3) To determine the mutagenic potential of unconcentrated and concentrated (10X) samples of the effluent using the Ames assay.

4) To determine teratogenic potential of the effluent using the frog (Xenopus laevis) embryo teratogenesis assay - Xenopus (FETAX).

5) To determine carcinogenic potential of the effluent using a a 6-month Japanese medaka (Oryzias latipes) test.

6) To test USABRDL's 21-day bluegill (Lepomis macrochirus) biological monitoring early warning system which can detect rapid changes in the acute toxicity of the effluent.

7) To quantify the major chemicals present in the effluent and monitor the general water quality of the effluent.

SECTION 3

MATERIALS AND METHODS

3.1 Background Information

The study was conducted on-site in USABRDL's Aquatic Biomonitoring Trailer Version 1.0. A complete description of the trailer layout, associated equipment and instrumentation, study protocols, etc., may be found in Herriott and Burton (1992). Briefly, the biomonitoring trailer is a specially designed 8 ft x 24 ft mobile laboratory which is divided into two compartments: a small room (8 ft x 5 ft) used primarily to isolate fish used in the ventilatory biological monitoring system and a two-tiered large room (8 ft x 19 ft) used for flow-through toxicity testing (e.g., teratogenicity and carcinogenicity) water quality testing, storage of test materials, and data acquisition. The trailer is supplied with a 240 volt (single phase), 100 amp power supply and a back-up generator.

APG-EA WWTP provided additional space at the plant for a water filtration system, aeration/equilibration tanks, water sampler, water pumps, air compressor, and bluegill acclimation space. Aberdeen dechlorinated potable water (charcoal filtered) which was used as diluent water and APG-EA WWTP effluent were supplied to the trailer via PVC pipe. Excess diluent water and effluent from the trailer were collected and returned to the plant for further treatment before being discharged.

Acute toxicity was evaluated daily on 24-h composite samples using the 15-min Microtox® assay which employed microbial (Photobacterium phosphoreum) bioluminescent activity. Two 24-h LC50 rotifer (Brachionus rubens) toxicity tests were conducted using 24-h composite samples. The following chronic tests were all performed two times using 24-h composite samples as described below: 96-h EC50 algal (Selenastrum capricornutum) growth test, 7-d daphnid (Ceriodaphnia dubia) survival and reproduction test, and 7-d fathead minnow (Pimephales promelas) survival and growth test. A summary of the sample periods for all tests is given in Table 1.

Six mutagenicity assays (Ames) were performed on the effluent and two on the diluent water using 24-h composite samples. One preliminary 96-h (flow-through) teratogenicity test was conducted using the African clawed frog (Xenopus laevis) embryo teratogenesis assay (FETAX). A preliminary 6-month carcinogenicity test was conducted under flow-through test conditions with Japanese medaka (Oryzias latipes) unexposed fry and fry initiated with diethylnitrosoamine (DEN). USABRDL's 21-d bluegill (Lepomis macrochirus) computerized ventilatory monitoring system was tested four times. Seven comprehensive chemical analyses were performed on 24-h composite samples of both the effluent and diluent water. Routine water quality was also determined frequently throughout the study period.

3.2 Acute Toxicity

3.2.1 Microtox® Test

The Microtox® test (Microbics Corp., Carlsbad, CA) is a rapid acute toxicity test that may be completed in less than one hour. The test is based on the reduction in bioluminescence of the marine bacterium P. phosphoreum when exposed to a sample of unknown toxicity. The degree of light reduction, an indication of metabolic inhibition in the test organisms, indicates the degree of toxicity of the sample. The Microtox® test procedures followed were those outlined in the Microtox® operating manual (Microtox®, 1988). A Microtox® Model 2055 Analyzer was used for all tests.

Microtox® tests were conducted from June 5, 1989 until the termination of the carcinogenicity study on December 12, 1989. Each Microtox® test was conducted on-site from an aliquot taken from a 24-h composite sample of 100% effluent collected by an Isco® refrigerated sampler (Model 2700R; Isco Inc., Lincoln, NE). A 15-min test was performed on all samples; no 5-min tests were conducted.

3.2.2 Rotifer Toxicity Test

The potential toxicity of the effluent was determined two times using the Rotifer Toxkit™ Screening Test (US TOXKIT, Tampa, FL). The test utilized newly hatched rotifers (B. rubens) <4 h old. The rotifers used in the tests were hatched from cysts supplied in the Rotifer ToxKit™. Rotifer ToxKit™ synthetic medium was used to hatch the cysts, rear the organisms before testing, and served as the control medium during the test. The static tests were conducted in glass Petri dishes containing 10 mL of test solution. All rotifer tests were conducted at The Johns Hopkins University Applied Physics Laboratory-Aquatic Ecology Section (JHU/APL-AES) Laboratory in Shady Side, MD.

Preliminary tests showed that 100% effluent was not acutely toxic; therefore, 100% APG-EA WWTP effluent only was tested in the first test. Although the effluent was not toxic in the first definitive test, a complete series of effluent concentrations was used in the second test to check for possible interactive effects at the lower concentrations. The effluent used in each test was taken from a 24-h composite sample which was collected in a refrigerated Isco® sampler (Model 2700R; Isco Co., Lincoln, NE). The effluent, which was used within 24 h from the time of collection, was held in glass containers at 4°C until used in the tests. Three replicates of 10 organisms each were performed at each test concentration. All tests were conducted at 25 ± 0.5°C. Routine water quality was taken at the beginning and end of each test. All tests were conducted under a 16-h light:8-h dark photoperiod (fluorescent lights at 60-85 foot candles).

3.3 Chronic Toxicity

All chronic tests were conducted at the JHU/APL-AES Laboratory using non-chlorinated deep well water as diluent water. A comprehensive chemical analysis of the JHU/APL-AES diluent water is given in Table 2. The effluent samples used in all tests were taken from 24-h composite samples which were collected in a refrigerated Isco® sampler (Model 2700R; Isco Co., Lincoln, NE). All effluent was transported to the laboratory in glass containers placed on ice and held at 4°C until used in the tests. One 24-h composite sample was used for each algal test within 24 h of collection. Three 24-h composite samples, which were collected, transported, and held as described above, were obtained on days 1, 3, and 5 of the 7-d tests with both the invertebrate and fish. Both the daphnid and fathead minnow tests were conducted using aliquots taken from the same effluent sample.

3.3.1 Green Algal Growth Test

A _S. capricornutum_ starter culture was obtained from the culture collection at North Texas State University, Denton, TX. Stock algal cultures were reared in 2.5 L Pyrex culture flasks containing 1 L of sterilized double strength "AAP" algal assay medium, with sufficient P added to achieve a 20:1 N:P ratio as described in Miller et al. (1978). Cultures were maintained in a constant temperature incubator under constant cool-white fluorescent lights (≈300 foot candles) at a temperature of 20 ± 1°C on a shaker table oscillating at 100 rpm (± 10 %). Log growth cells were used to start all tests.

The potential toxicity (96-h EC50 for growth) of the effluent to _S. capricornutum_ was determined two times (Table 1) by the procedures given in Horning and Weber (1985). The nutrient media used for the bioassays was sterilized double strength "AAP" algal assay medium, with sufficient P added to achieve a 20:1 N:P ratio as described in Miller et al. (1978) rather than the media recommended in the test method.

Algal test solutions were prepared by dilution of the effluent with filtered sterilized assay media within a sterile transfer room. Test solutions (100 mL total volume) were dispensed into 250 mL Delong flasks and inoculated with _S. capricornutum_ cells in log growth to achieve a density of ≈5 x 10^3 cell/mL. Triplicates were prepared for each treatment. The flasks were placed on a shaker table in an incubator set at the culturing conditions described above. Growth measurements (cell density) were made from all replicates in each treatment at 0, 24, 48, 72, and 96 h. Algal cell density was determined from a 1 mL sample with a Model ZBI Coulter Counter (Coulter Electronics Inc., Hialeah, FL). The instrument was calibrated with each use via hemocytometer counts.

3.3.2 Daphnid Survival and Reproduction Test

The cladoceran, C. dubia, was cultured at 25 ± 1°C in 600 mL glass beakers filled with 400 mL JHU/APL-AES well water amended with selenium (2 ug Se/L as Na_2SeO_3) as recommended by Winner (1987 and 1989). The diet consisted of a mixture of Cerophyl® (Cerophyl Laboratories, Inc., Kansas City, MO) and the green alga, S. capricornutum, added to the daphnid culture to achieve final concentrations of 120 ug Cerophyl®/mL and 6.7 x 10^5 S. capricornutum cells/mL. Starter cultures of C. dubia were obtained from the Center for Lake Superior Environmental Studies, University of Wisconsin - Superior.

The chronic toxicity of the effluent to Ceriodaphnia was determined two times (Table 1) by the method given in Draft No. 3 of the ASTM proposed guide for conducting three brood, renewal toxicity tests (Waller and Lazorchak, 1986). All neonates used in the 7-d survival and reproduction tests were produced by daphnids in culture that had released at least three broods. The initial age of the neonates in each test was <24 h old. The tests were conducted in 50 mL glass beakers containing 30 mL of test solution. All tests were conducted in an environmental chamber at 25 ± 1°C under a 16-h light:8-h dark photoperiod (fluorescent lights; 60-85 foot candles at the surface of the culture vessels). All test organisms were fed daily as described above at each 24-h renewal. Routine water chemistry was taken at each renewal.

3.3.3 Fathead Minnow Survival and Growth Test

Fathead minnow (P. promelas) larvae, <24 h at the start of the tests, were obtained from the JHU/APL-AES culture maintained at 25 ± 1°C in JHU/APL-AES well water. The JHU/APL-AES culture procedures were similar to those recommended by Peltier and Weber (1985). The JHU/APL-AES culture was initiated with mature fathead minnows obtained from the U.S. EPA Environmental Monitoring and Support Laboratory - Cincinnati, Ohio. Briefly, spawning fish were cultured in fiberglass tanks (2.4 x 0.8 x 0.5 m) containing 0.2 m JHU/APL-AES well water held at 25 ± 1°C. The spawning adults were fed a diet of frozen brine shrimp (Artemia sp.; Argent Chem. Lab., Redmond, WA) and TetraMin® Staple Food (Ramfab Aquarium Products Co., Oak Ridge, TN) twice daily. Excess food was removed daily. Five sets of spawning fathead minnows were maintained in the culture tanks at a ratio of 1 male:3 females. Replacement spawners were rotated at approximately 3-month intervals. Fathead minnow embryos were collected on spawning substrates (10 cm I.D. x 20 cm long PVC pipe sections cut longitudinally in equal portions) and transferred to 19 L aquaria at 25 ± 1°C in JHU/APL-AES well water for hatching. All stages of the fish were reared under a 16-h light:8-h dark photoperiod (fluorescent lights; 60-85 foot candles).

The chronic toxicity of the effluent to fathead minnows was determined two times (Table 1) by the static renewal method (solutions renewed daily) given in Weber et al. (1989). All larvae used in the 7-d survival and growth tests were <24 h old. The tests were conducted in 600 mL glass beakers containing 500 mL of test solution. All test organisms were fed brine shrimp (Artemia sp.) nauplii <24 h old daily at each 24-h renewal. All tests were conducted at 25 ± 1°C under a 16-h light:8-h dark photoperiod (fluorescent lights; 60-85 foot candles). Routine water chemistry was taken at each renewal. Dry weight was determined by drying at 100°C for a minimum of 12 h.

3.4 Mutagenicity

Salmonella/mammalian-microsome reverse mutation assays (Ames test) were conducted six times on APG-EA WWTP effluent and on two APG diluent water samples (Table 1). Ames assays were conducted on both unconcentrated and concentrated (10X via XAD-2 resin extracts) samples of the effluent and diluent water. The Ames mutagenicity assays were conducted by Hazleton Laboratories America, Inc., Kensington, MD.

Composite samples (24 h) of effluent were collected in 45 L (12 gallon) polypropylene containers packed in ice by Isco™ samplers (Model 2700; Isco Inc., Lincoln, NE). Grab samples of diluent water were collected in a large polypropylene tank with a 99% particle replacement time of ≈12 h. Thirty-one liters (1 L for the unconcentrated sample and 30 L for the 10X sample) of each material were siphoned into appropriately labeled 1 L Nalgene polycarbonate bottles, packed in ice, and transported to Hazleton Laboratories America, Inc., in insulated containers. All unconcentrated samples of effluent and diluent water were analyzed by Hazleton Laboratories America, Inc. Protocol No. HLA Protocol 401W, Edition 16. All concentrated (10X) samples were analyzed by Protocol No. HLA Protocol 401X, Edition 16. Effluent and diluent water were also taken during the same sampling period for detailed chemical analyses (see Section 3.8.1).

The experimental procedures for the unconcentrated and 10X tests are given in the protocols shown above. Briefly, the mutagenicity assays evaluated the effluent and diluent water samples for their ability to induce reverse mutations at the histidine locus in the genome of specific S. typhimurium tester strains both in the presence and absence of an exogenous metabolic activation system of mammalian microsomal enzymes derived from Aroclor 1254-induced rat liver. The tester strains used in the assays were TA98 and TA100. The assays were conducted using two plates per dose level in the presence of microsomal enzymes. Six dose levels of the effluent and diluent water samples were tested in both the presence and absence of S9 along with appropriate vehicle controls (three plates per dose), negative controls, and positive controls. Resin controls were also run for the 10X samples. The doses tested in the 10X assays varied based on the amount of extractable organics recovered from the test material.

3.5 Teratogenicity

One preliminary teratogenicity test was conducted from September 25-29, 1989 using the frog embryo teratogenesis assay - Xenopus (FETAX) which is a 96-h quantitative teratogen assay used to screen for developmental toxicants in aquatic media. The preliminary FETAX assay was conducted under flow-through test conditions during the 6-month continuous exposure carcinogenicity test. The assay was conducted by the method given in Draft No. 2 of the ASTM proposed guide for conducting FETAX (Bantle and Sabourin, 1989) with the following exception. The ASTM method states that five test concentrations plus controls should be used. However, only two flow-through effluent concentrations (100% effluent and 10% effluent by volume) plus controls were available in the mobile trailer because the FETAX tests were run in the same flow-through system used for the 6-month carcinogenicity test (see Section 3.6).

Embryos between normal stage 8 blastulae and normal stage 11 gastrulae were obtained from Xenopus breeding colonies at USABRDL. The embryos were suspended in FETAX solution in an Erlenmeyer flask and delivered to the trailer on the morning the test was initiated by USABRDL personnel. The embryos were de-jellied with 200 mL of a 2% L-cysteine solution (2 g of L-cysteine per 98 mL of FETAX solution). Once de-jellied, the embryos were rinsed and re-suspended in FETAX solution. The embryos were placed in twelve 250 mL mesh bottomed glass beakers (25 embryos/beaker) which were suspended by a wire harness (1 beaker per aquarium) in the 5 gallon aquaria used in the 6-month carcinogenicity test (4 aquaria at 100% effluent; 4 at 10% effluent by volume; and 4 diluent water controls). The tests were conducted at 25 ± 1°C under a 16-h light: 8-h dark photoperiod (fluorescent lights; ≈75 foot candles).

The beakers were checked daily for mortality. At the end of the 96-h exposure, the organisms were anesthetized using MS-222 and subsequently fixed in a 3% formalin solution. All organisms were sent to USABRDL for morphological analysis by their FETAX staff.

3.6 Carcinogenicity

The Japanese medaka (*O. latipes*), which has been shown to be a sensitive laboratory carcinogen model (for ex., see Hawkins et al., 1988; Klaunig et al., 1984; Metcalfe, 1989), was used to screen for environmental pollutants which may induce neoplasms. Both unexposed and fry initiated with diethylnitrosoamine (DEN) were used in a preliminary 6-month continuous exposure test conducted in the mobile laboratory at the APG-EA WWTP from June 14, 1989 to December 13, 1989. The test was given the designation Carcinogenicity Test (O) by USABRDL.

Two test concentrations (100% effluent and 10% effluent by volume) plus APG diluent water (control) were used in the study. The test solutions were delivered by a solenoid-activated proportional dilutor system which was constructed primarily of glass and stainless steel; silicon tubing was also used. The test concentrations were delivered to twelve 19 L (5 gallon) aquaria (4 aquaria at 100% effluent; 4 at 10% effluent by volume; and 4 control aquaria); each aquarium contained a volume of ≈16 L (4.25 gallons). All aquaria were held in a constant temperature (25 ± 1°C) water bath. The dilutor was calibrated to complete one full cycle every 2.5-3.5 minutes. During a cycle, tanks 1-4 received 250 ± 50 mL of diluent water, tanks 5-8 received 250 ± 50 mL of 10% effluent by volume, and tanks 9-12 received 250 ± 50 mL of 100% effluent.

Both unexposed fry and fry (14-d old) exposed to 20 mg/L DEN for 48 h were reared off-site at USABRDL, Ft. Detrick, Frederick, MD until 26 days old. At 26 d old, the fish were taken to the biomonitoring trailer at APG-EA WWTP and suspended in twelve 1000 mL mesh-bottom glass beakers in appropriate flow-through test aquaria in the mobile laboratory. The fish were held in the beakers for one week after which they were released into the aquaria.

Pre-adult fish, 26-30 days old, were fed Tetramin® flake food (2 feedings per day Monday, Wednesday, Friday, Saturday, and Sunday; and 1 feeding per day Tuesday and Thursday), live brine shrimp <48 h old (1 feeding per day, 40 brine shrimp per fish), and ground ocean plankton (Silco Pet Products Co., Alexandria, VA) (1 feeding per day Tuesday and Thursday). Adult fish, 31 days or older, were fed Tetramin® flake food (2 feedings per day Monday through Friday and 1 feeding per day Saturday and Sunday), live brine shrimp (1 feeding per day Monday, Wednesday, and Friday), and ground ocean plankton (1 feeding per day Tuesday, Thursday, Saturday, and Sunday). Tanks were cleaned on an as needed basis (usually 1-2 times a week) by scrubbing algae from the sides of the tanks, allowing the debris to settle, and then siphoning. Tetramin® and ground ocean plankton were fed *ad libitum* for 15-30 min during each feeding.

The number of test organisms alive in each tank was monitored and recorded daily. Dead or moribund fish were fixed for subsequent pathological observation. The dilutor cycle time was calculated and recorded daily. The volume of effluent and diluent water delivered to the aquaria was checked weekly. When necessary, cycle time and/or volume distributions were adjusted. The dilutor was shutdown (<1 h) and cleaned on an as needed basis. Routine water quality was determined once each week in one aquarium which contained 100% effluent and one diluent water aquarium (see Section 3.8.2).

On day 91 of the exposure period, 20 Japanese medaka in each tank were killed, fixed, and taken back to USABRDL for pathological observation. Approximately 15 additional fish from each tank were taken back to USABRDL for recovery observations. On day 183, when the exposure was completed, the remaining Japanese medaka were also taken back to USABRDL for recovery observations and subsequent pathological analysis.

3.7 Biological Monitoring Early Warning System

The 21-d bluegill (L. macrochirus) computerized ventilatory monitoring system, which is a real-time continuous monitoring system, was run in a field test mode to detect possible unexpected abrupt changes in effluent quality or episodic events which may be harmful to the aquatic environment. The system uses changes in fish ventilation frequency, opercular amplitude, and cough frequency to predict acute toxicological effects (Shedd et al., 1986). Individual fish in two control and two experimental groups of 8 fish/group (total of 32 fish) are held in the test system for a period of 21 days during a typical ventilatory test. The 21-d period includes an initial 3-d "acclimation" period (no data are collected during the 3-d period) followed by a 4-d period in which all 32 fish receive diluent water only in order to establish baseline data. At the end of the baseline period, two groups of 8 fish/group are switched to effluent for 14 d of monitoring while exposed to effluent. The fish are isolated from all activity including feeding during the 21-d period.

Four ventilatory tests were performed during the APG-EA WWTP study (Table 1). Preliminary toxicity tests with the bluegill showed that the effluent was not acutely toxic; therefore, 100% effluent was used as the test concentration. APG-EA WWTP effluent and APG dechlorinated diluent water were supplied to a four component ventilatory dilutor system which was calibrated to complete one full cycle every 55-65 seconds. During a cycle, 16 ventilatory chambers received 50 ± 2.5 mL of effluent, while the remaining 16 ventilatory chambers received 50 ± 2.5 mL of diluent water. A complete description of the ventilatory diluter system, components of the data acquisition system, etc., is given in Herriott and Burton (1992). Information concerning the software of the data acquisition system, acquisition of the automated water quality, etc. may be found in USABRDL (1991).

Juvenile bluegills (6.4-9.0 cm standard length; 2.5-3.5 inches) were reared off-site at USABRDL. Two weeks prior to each test, bluegills were delivered to the APG-EA WWTP study site for acclimation in APG diluent water. The fish were fed trout chow or frozen brine shrimp twice daily. Dead or moribund fish were removed and disposed of immediately to reduce the risk of disease to the other bluegills. Tanks were siphoned of debris daily and thoroughly cleaned once a week. The fish were held at 25 ± 2°C under continuous light (fluorescent lights; ≈75 foot candles).

On day 1 of the test, 32 bluegills were randomly transferred to 32 ventilatory chambers. Once placed in the ventilatory chambers, the fish were oriented to face the water input end of the test chamber. The ventilatory chambers were then connected to their designated leads to the biomonitoring data acquisition system. Signals from each test chamber were checked via an oscilloscope for clarity before initiating the test.

Computer and printer operation were checked daily. Entry into and exit from the biomonitoring trailer were recorded each time the event occurred. When entering and exiting the trailer, the computer screen was printed along with the entry or exit time. In addition, any unusual events (e.g., external noise, low DO, reduced water pressure) were noted during their occurrence. These data were collected to eliminate possible false events during a ventilatory run. The ventilatory signal of each fish was checked daily via an oscilloscope and the data acquisition system.

The cycle times of dilutors 1-4 were measured, calculated, and recorded daily. When necessary, cycle times were adjusted. The high and low electrodes located in each mixing chamber were inspected daily and cleaned on an as needed basis. Aeration was performed in the 100% effluent mixing chambers to increase DO concentrations. All solenoids and delivery lines were inspected daily to ensure that they were operating properly.

Routine water quality was measured via grab samples taken from a dilutor flow splitting cup containing 100% effluent and one containing diluent water as described in Section 3.8.2. Water quality was also monitored continuously and logged on the data acquisition system as described in Section 3.8.4.

At the end of a test all bluegills were weighed (wet weight) and measured (total length). The volume of effluent or diluent water delivered to each ventilatory chamber was measured and recorded. The data from each test were transferred from the data acquisition system to floppy disks for subsequent analysis at USABRDL.

3.8 Chemical Analyses

3.8.1 Comprehensive Chemical Analyses

Comprehensive chemical analyses were performed seven times on 24-h composite samples of APG-EA WWTP effluent and APG dechlorinated tap water by Biospherics Inc. (Beltsville, MD) as shown in Table 1. APG-EA WWTP effluent (11 L) was collected in a 45 L (12 gallon) polypropylene container (submerged in an ice bath) by an Isco® sampler (Model 2700; Isco Inc., Lincoln, NE). The effluent was then siphoned into several containers provided for various analyses. The containers were placed on ice and delivered to Biospherics Inc. for analysis. Grab samples of diluent water were taken from a large polypropylene tank with a 99% particle replacement time of ≈12 h, placed in appropriate containers, and delivered on ice to Biospherics Inc. for analysis.

The materials analyzed in the effluent and diluent water and their quantitation limits are listed in Table 3. The analytical methods used by Biospherics Inc. for general water chemistry, metals, volatiles, semi-volatiles, PCB/pesticides, and herbicides for both the diluent water and effluent are given in Table 4.

3.8.2 Routine Water Quality Analyses

Routine water quality analyses were conducted once a week on grab samples taken from two carcinogenicity test aquaria and from the ventilatory dilutors in the biological monitoring early warning system as described in Sections 3.6 and 3.7, respectively. The following water quality parameters were measured: temperature, pH, dissolved oxygen, alkalinity, and hardness. The analytical methods are summarized in Table 5.

In addition to the temperature measurements made via grab samples during the carcinogenicity test, temperature was monitored continuously in the water bath which held the exposure aquaria via a strip chart recorder (Cole-Parmer Thermistor Recorder Model No. 08354-15, Cole-Palmer Instrument Co., Chicago, IL). Temperature was also monitored continuously during each ventilatory test from 1) a thermistor placed in one of the ventilatory dilutor chambers and transduced to a strip chart recorder (same model as above) and 2) via the data acquisition system described below in Section 3.8.4.

3.8.4 Automated Water Quality Analyses

The following water quality parameters were continuously monitored at 30-min intervals during the 21-d ventilation studies for both the effluent and diluent water: DO, pH, temperature, conductivity, and turbidity. The ventilatory data acquisition system was programmed to record a 30 min average measurement of each parameter in the effluent followed by a 30 min average measurement of the parameters in the diluent water. A Hydrolab® Scout® (Hydrolab Corp., Austin, TX) was used to monitor DO, pH, temperature, and conductivity. A Hach® Surface Scatter 5 Turbidimeter (Hach Co., Loveland, CO) was used to monitor turbidity. As was the case for the ventilation data discussed in Section 3.7, the water quality data from each test were also transferred from the data acquisition system to floppy disks for subsequent analysis at USABRDL.

SECTION 4

RESULTS AND DISCUSSION

4.1 Acute Toxicity

4.1.1 Microtox®

The results of the Microtox® tests conducted from June 5, 1989 to December 12, 1989 are summarized in Table 6. Acute toxicity was found in ≈3% of the effluent samples analyzed (toxicity occurred 3 days out of 95 days of sampling). The three 15-min EC50s ranged from 1.1 to 18.8 percent effluent by volume. The three days of acute toxicity occurred during the period July 20-26, 1989. Three other positive tests were rejected during the study because the EC50s were >100% effluent by volume. The >100% EC50s occurred August 6-8, 1989 when problems developed with the Microtox® instrument.

4.1.2 Rotifer Toxicity Test

The effluent (100% effluent) was not acutely toxic to the rotifer in two separate tests. A synopsis of each test performed, mean water quality, and rotifer survival are given in Appendices 1 and 2.

4.2 Chronic Toxicity

4.2.1 Green Algal Growth Test

No toxicity occurred in the tests conducted with the green alga during the periods July 25-29, 1989 and October 29 to November 2, 1989 (see Appendices 3 and 4). The effluent caused a slight stimulation of algal growth relative to the controls in the first test (Appendix 3; Table A3-2). A synopsis of each test performed, cell density, growth rate, etc., are given in Appendices 3 and 4.

4.2.2 Daphnid Survival and Reproduction Test

APG-EA WWTP effluent had no affect on adult survival or neonate production at concentrations up to 100% effluent by volume in the first test conducted June 15-22, 1989 (Appendix 5). The effluent did not affect the survival of the adults after 7 d of exposure in the second test conducted October 30 to November 6, 1989; however, a statistically significant (alpha = 0.05) increase in neonate production (relative to the controls only) occurred at all effluent concentrations tested (Appendix 6; Tables A6-2 and A6-3). The lower neonate production in the controls may be attributable to statistical chance, i.e., 1 in 20 times one can expect a random event to occur. A synopsis of each test performed, mean water quality, adult survival, neonate production, and statistical analysis of the data are given in Appendices 5 and 6.

4.2.3 Fathead Minnow Survival and Growth Test

The first fathead minnow test (June 14-21, 1989) was rejected because >20% mortality occurred in the controls (Appendix 7). Although the test was rejected, the data indicate that the effluent did not cause greater mortality in any treatment group (relative to the controls) up to 100% effluent by volume. In fact, survival was greater in all treatment groups than the control groups.

The effluent had no affect on larval survival at concentrations up to 100% during the second test conducted October 27 to November 3, 1989 (Appendix 8). However, the effluent did affect the growth of the larvae. A statistically significant (alpha = 0.05) reduction in growth occurred in the 50% effluent by volume treatment only (Appendix 8; Tables A8-2 and A8-3). It is not clear why a reduction in growth occurred in the 50% effluent by volume treatment when a reduction did not occur in the 100% effluent by volume treatment.

4.3 Mutagenicity

The results of the Ames mutagenicity assays conducted during the APG-EA WWTP study are summarized in Table 7. None of the unconcentrated effluent or diluent water samples caused a positive increase in the numbers of histidine revertants per plate with tester strains TA98 or TA100 either in the presence or absence of microsomal enzymes prepared from Aroclor 1254-induced rat liver, that is, no mutagenic activity was found. Similarly, the two concentrated (10X) diluent water samples had no mutagenic activity. All six concentrated (10X) effluent samples had mutagenic activity.

The concentrated (10X) APG-EA WWTP effluent sample taken on July 24, 1989 caused a positive increase (2.0-fold) in the number of histidine revertants per plate with tester strain TA100 in the absence of microsomal enzymes prepared from Aroclor 1254-induced rat liver. The concentrated effluent sample taken on September 7, 1989 caused a reproducible positive increase (2.1 and 2.2-fold) in the number of TA98 revertants per plate in the presence of microsomal enzymes prepared from Aroclor 1254-induced rat liver (S9). In the absence of S9, a positive increase was observed in only one of two trials with tester strain TA98 (2.4 and 1.9-fold). No positive increases were observed with tester strain TA100 either in the presence or absence of S9.

The results of the Ames test conducted on the September 28, 1989 effluent concentrated 10X showed that reproducible positive increases (2.4 and 2.3-fold) occurred in the number of histidine revertants per plate with tester strain TA98 in the absence of S9, and with tester strain TA100 (2.2 and 2.2-fold) in the absence of S9. A positive response was observed in only one of two trails with tester strain TA98 in the presence of S9 (2.3 and 1.8-fold). The 10X effluent sample of October 26, 1989 caused reproducible positive increases (3.1 and 4.0-fold) in the number of histidine revertants per plate with tester strain TA98 in the presence of microsomal enzymes prepared with Aroclor 1252-induced rat liver.

The concentrated effluent sample of November 16, 1989 caused a positive increase in the number of histidine revertants per plate with tester strain TA98 (2.4 and 2.1-fold) only in the presence of microsomal enzymes prepared from Aroclor 1252-induced rat liver. None of the remaining tester strain/activation conditions produced positive responses. The December 7, 1989 10X effluent caused a positive increase in the number of histidine revertants per plate with tester strain TA98 (2.7 and 2.5-fold) only in the presence of Aroclor 1254-induced rat liver microbial enzymes. No positive increases were observed with tester strain TA98 in the absence of S9 or with tester strain TA100 in either the presence or absence of S9.

4.4 Teratogenicity

No data are available for the FETAX assay conducted during the APG-EA WWTP study because the assay was a preliminary assay.

4.5 Carcinogenicity

No data are available from Carcinogenicity Test (O) because the test was a preliminary test.

4.6 Biological Monitoring Early Warning System

No abrupt changes in effluent quality or episodic events were detected during Ventilatory Tests I (June 5-26, 1989), II (July 11 to August 1, 1989) or IV (October 20 to November 7, 1989). The effluent was not overtly toxic to the bluegills during the 14-d definitive phases of each test. That is, significant mortality did not occur during 14 d of exposure to 100% effluent. A number of plant operation problems occurred during Ventilatory Test III which ultimately caused the test to be terminated before it was completed. During Ventilatory Test III, the system detected both an increase in sediment load and chlorine concentration during separate episodes. Mr. Tommy R. Shedd of USABRDL may be contacted for further information concerning ventilation frequency, opercular amplitude, cough frequency, etc., obtained during the studies.

4.7 Chemical Analyses

4.7.1 Comprehensive Chemical Analyses

The results of the four comprehensive chemical analyses of the APG-EA WWTP effluent and APG diluent water are summarized in Table 8. The only values reported are for those chemicals whose concentrations were at or above the quantitation limits given in Table 3. Copper, one of eight heavy metal priority pollutants (Section 307 toxic pollutants) measured in this study, was detected in both the effluent and diluent water sample taken November 16, 1989. Of the eight heavy metal priority pollutants measured (Note: there are 12 heavy metal priority pollutants), no other heavy metal priority pollutant was detected in the effluent or diluent water.

Chloroform, a priority pollutant, was detected in both the effluent and diluent water on four occasions (Table 8). 1,1,1-Trichloroethane was detected once in the diluent water. Di-n-octyl phthalate was also detected once in the diluent water only.

4.7.2 Routine Water Quality Analyses

The raw water quality and a statistical summary of the data in the two carcinogenicity test aquaria for the period June 18, 1989 to December 11, 1989 are summarized in Table 9. Briefly, the mean temperature, pH, dissolved oxygen, alkalinity, and hardness of the effluent was 24.8°C, 6.8, 7.3 mg/L, 50 mg/L as $CaCo_3$, and 108 mg/l as $CaCo_3$, respectively. The mean temperature, pH, dissolved oxygen, alkalinity, and hardness of the diluent water was 24.8°C, 6.9, 7.7 mg/L, 41 mg/L as $CaCo_3$, and 84 mg/l as $CaCo_3$, respectively. Mr. Tommy R. Shedd of UASBRDL may be contacted for the routine water quality data taken via grab samples during the biological monitoring early warning system tests.

4.7.3 Automated Water Quality Analyses

Mr. Tommy R. Shedd of UASBRDL may be contacted for the automated routine water quality data logged during the four biological monitoring early warning system tests.

SECTION 5

CONCLUSIONS

The array of biological monitoring techniques used to assess the potential toxicity of the APG-EA WWTP effluent showed that the effluent generally was not toxic during most of the study period. Toxicity was detected by the following test systems. Acute toxicity was found in ≈3% of the effluent samples measured (toxicity occurred 3 days out of 95 days of sampling) via Microtox®. Three 15-min EC50s, which ranged from 1.1 to 18.8% effluent by volume, occurred during the period July 20-26, 1989. No acute toxicity was found in the 24-h rotifer tests.

No significant chronic toxicity was detected by the three biomonitoring systems used during two separate sets of tests. The effluent was not toxic to the green alga or the daphnid. A statistically significant (alpha = 0.05) reduction in fathead minnow larval growth occurred in the 50% effluent by volume treatment only during the second test. However, it is not clear why a reduction in growth occurred in the 50% effluent by volume treatment when a reduction did not occur in the 100% effluent by volume treatment. The reduction in growth at 50% effluent by volume may be attributable to statistical chance, i.e., 1 in 20 times one can expect a random event to occur.

No mutagenicity was detected in unconcentrated APG-EA WWTP effluent or unconcentrated dechlorinated APG diluent water. All concentrated (10X) effluent samples had mutagenic activity. No teratogenicity data are available because the test was a preliminary study. No data are available from Carcinogenicity Test (O) because the test was a preliminary test.

No abrupt changes in effluent quality or episodic events were detected during Ventilatory Tests I, II, or IV. The effluent was not overtly toxic to the bluegills during the 14-d definitive phases of each test. That is, significant mortality did not occur during 14 d of exposure to 100% effluent. A number of plant operation problems occurred during Ventilatory Test III which ultimately caused the test to be terminated before it was completed. During Ventilatory Test III, the system detected both an increase in sediment load and chlorine concentration during separate episodes.

SECTION 6

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TABLE 1. SUMMARY OF THE BIOMONITORING TESTS CONDUCTED.

Test and/or Species	Type of Test	Test Periods	Comments
Microtox® (Bacterium)	15-min EC50	06/05/89 - 12/12/89	Periodic testing of 24-h composite samples
ToxKit™ (Rotifer)	24-h LC50	06/21/89 - 06/23/89 10/31/89 - 11/02/89	24-h composite sample
Green alga	96-h EC50	07/25/89 - 07/29/89 10/29/89 - 11/02/89	24-h composite sample
Daphnid	7-d Survival and reproduction	06/15/89 - 06/22/89 10/05/89 - 10/12/89	24-h composite samples renewed every 24 h
Fathead minnow	7-d Survival and growth	06/14/89 - 06/21/89 10/27/89 - 11/03/89	24-h composite samples renewed every 24 h
Mutagenicity (Bacterium)	Ames assay	07/24/89 09/07/89 09/28/89 10/26/89 11/16/89 12/07/89	24-h composite sample
Teratogenicity (African frog)	4-d FETAX	09/25/89 - 09/29/89[a]	Flow-through exposure
Carcinogenicity (Japanese medaka)	6 months	06/14/89 - 12/13/89[a]	Flow-through exposure

TABLE 1. (CONTINUED).

Test and/or Species	Type of Test	Test Periods	Comments
Bluegill early warning system	21 d	06/05/89 - 06/26/89 07/11/89 - 08/01/89 08/11/89 - 09/01/89 10/20/89 - 11/07/89	Flow-through exposure
Comprehensive chemical analyses	N/A	06/21/89 07/24/89 09/07/89 09/28/89 10/26/89 11/16/89 12/07/89	24-h composite sample
Routine water quality analyses	N/A		See text

* Preliminary test only.

TABLE 2. COMPREHENSIVE WATER CHEMISTRY ANALYSIS OF THE JHU/APL-AES WELL WATER.

Base/Neutrals

Compound	ug/L[a]	Compound	ug/L[a]
Bis(2-chloroethyl)ether		Di-n-butylphthalate	
1,3-Dichlorobenzene		Flouranthene	
1,4-Dichlorobenzene		Pyrene	
1,2-Dichlorobenzene		Butylbenzylphthalate	
Bis(2-chloroisopropyl)ether		3,3'-Dichlorobenzidine	
N-Nitroso-di-n-propylamine		Benzo(a)anthracene	
Hexachloroethane		Bis(2-ethylhexyl)phthalate	
Nitrobenzene		Chrysene	
Isophorone		Di-n-octylphthalate	
Bis(2-chloroethoxy)methane		Benzo(b)fluoranthene	
1,2,4-Trichlorobenzene		Benzo(k)fluoranthene	
Naphthalene		Benzo(a)pyrene	
Hexachlorobutadiene		Indeno(1,2,3-cd)pyrene	
Hexachlorocyclopentadiene		Dibenzo(a,h)anthracene	
2-Chloronaphthalene		Benzo(g,hi,i)perylene	
Dimethylphthalate			
Acenaphthylene		The following are non-priority pollutant hazardous substance list compounds:	
Acenaphthene			
2,4-Dinitrotoluene			
2,6-Dinitrotoluene		Aniline	
Diethylphthalate		Benzyl Alcohol	
4-Chlorophenyl-phenylether		4-Chloroaniline	
Fluorene		2-Methylnaphthalene	
N-Nitrosodiphenylamine		2-Nitroaniline	
4-Bromophenyl-phenylether		3-Nitroaniline	
Hexachlorobenzene		Dibenzofuran	
Phenanthrene		4-Nitroaniline	
Anthracene			
		DETECTION LIMIT	2

TABLE 2. (CONTINUED).

Pesticides		Metals	
Compound	ug/L[a]	Metal (Total)	mg/L
Alpha-BHC		Antimony	<0.005
Beta-BHC		Arsenic	<0.005
Delta-BHC		Beryllium	<0.005
Gamma-BHC (Lindane)		Cadmium	<0.001
Heptachlor		Chromium	<0.05
Aldrin		Copper	<0.02
Heptachlor epoxide		Mercury	<0.0002
Alpha-endosulfan		Lead	<0.005
Dieldrin		Nickel	<0.20
4,4'-DDE		Selenium	<0.005
Endrin		Silver	<0.01
Beta-endosulfan		Thallium	<0.005
4,4'-DDD		Zinc	<0.04
Endrin aldehyde			
Endosulfan sulfate		Water Quality	mg/L
4,4'-DDT			
Methoxychlor		Alkalinity (as $CaCO_3$)	156
Chlordane		Ammonia (as N)	0.15
Toxaphene		Hardness (as $CaCO_3$)	190
Aroclor 1016		Nitrate	<0.10
Aroclor 1221		Nitrite	<0.10
Aroclor 1232		Total Kjeldahl Nitrogen	0.15
Aroclor 1242		Total Organic Carbon	19
Aroclor 1248			
Aroclor 1254		pH	7.8
Aroclor 1260			
DETECTION LIMIT	0.1		

[a] Concentrations less than the detection limit are left blank.

TABLE 3. APG-EA WWTP EFFLUENT AND APG DILUENT WATER CHEMICAL CHARACTERISTICS AND THEIR QUANTITATION LIMITS - GENERAL WATER QUALITY.

Parameter	Quantitation Limits (mg/L)
Alkalinity (as $CaCO_3$)	5.0[a]
Ammonia (as N)	0.02
Cyanide	0.01
Hardness (as $CaCO_3$)	5.0[b]
Nitrite	0.02[c]
Nitrate	0.04[d]
Nitrate/nitrite combined as N	0.4
Phosphorous	0.02[e]
Sulfide	2.0[a]
Conductivity (umho/cm)	1.0
Total suspended solids	5.0[f]
Fluoride	0.10
Sulfate	1.0[g]
Chloride	1.0

TABLE 3. (CONTINUED) - METALS.

Parameter	Quantitation Limits (mg/L)
Aluminum	0.2[h]
Arsenic	0.01
Barium	0.2[i]
Beryllium	0.005
Boron	0.05[j]
Cadmium	0.005
Calcium	1.0[k]
Cobalt	0.05
Copper	0.02[l]
Iron	0.10
Lead	0.005[m]
Magnesium	1.0[n]
Manganese	0.02[o]
Mercury	0.0005
Molybdenum	0.05[p]
Nickel	0.04
Potassium	0.5[q]
Selenium	0.005
Silver	0.01
Sodium	1.0

TABLE 3. (CONTINUED) - VOLATILE ORGANICS.

C.A.S. Number	Compound Name	Quantitation Limits (ug/L)[r]
74-87-3	Chloromethane	10.0
74-83-9	Bromomethane	10.0
75-01-4	Vinyl chloride	10.0
75-00-3	Chloroethane	10.0
75-09-2	Methylene chloride	5.0
67-64-1	Acetone	100.0[s]
75-69-4	Trichlorofluoromethane	5.0[t]
75-15-0	Carbon disulfide	5.0[s]
107-02-8	Acrolein	50.0[t]
107-13-1	Acrylonitrile	50.0[t]
75-35-4	1,1-Dichloroethene	5.0
75-34-3	1,1-Dichloroethane	5.0
540-59-0	Trans-1,2-dichloroethene	5.0
67-66-3	Chloroform	5.0
107-06-2	1,2-Dichloroethane	5.0
78-93-3	2-Butanone	100.0[s]
71-55-6	1,1,1-Trichloroethane	5.0
56-23-5	Carbon tetrachloride	5.0
108-05-4	Vinyl acetate	50.0[s]
75-27-4	Bromodichloromethane	5.0
78-87-5	1,2-Dichloropropane	5.0
10061-01-5	Cis-1,3-dichloropropene	5.0
79-01-6	Trichloroethene	5.0
124-48-1	Dibromochloromethane	5.0
79-00-5	1,1,2-Trichloroethane	5.0
71-43-2	Benzene	5.0
10061-02-6	Trans-1,3-dichloropropene	5.0
110-75-8	2-Chloroethylvinylether	10.0[t]
108-10-1	4-Methyl-2-pentanone	50.0[s]
591-78-6	2-Hexanone	50.0[s]
75-25-2	Bromoform	5.0
127-18-4	Tetrachloroethene	5.0
79-34-5	1,1,2,2-Tetrachloroethane	5.0
108-88-3	Toluene	5.0
108-90-7	Chlorobenzene	5.0
100-41-4	Ethylbenzene	5.0
100-42-5	Styrene	5.0[s]
1330-20-7	Total xylenes	5.0[u]

TABLE 3. (CONTINUED) - SEMI-VOLATILE ORGANICS.

C.A.S. Number	Compound Name	Quantitation Limits (ug/L)
62-75-9	N-Nitrosodimethylamine	10.0
108-95-2	Phenol	10.0
111-44-4	Bis(-2-chloroethyl)ether	10.0
95-57-8	2-Chlorophenol	10.0
541-73-1	1,3-Dichlorobenzene	10.0
106-46-7	1,4-Dichlorobenzene	10.0
100-51-6	Benzyl alcohol	10.0
95-50-1	1,2-Dichlorobenzene	10.0
95-48-7	2-Methylphenol	10.0
39638-32-9	Bis(2-chloroisopropyl)ether	10.0
106-44-5	4-Methylphenol	10.0
621-64-7	N-Nitroso-di-n-propylamine	10.0
67-72-1	Hexachloroethane	10.0
98-95-3	Nitrobenzene	10.0
78-59-1	Isophorone	10.0
88-75-5	2-Nitrophenol	10.0
105-67-9	2,4-Dimethylphenol	10.0
65-85-0	Benzoic acid	50.0
111-91-1	Bis(-2-chloroethoxy)methane	10.0
120-83-2	2,4-Dichlorophenol	10.0
120-82-1	1,2,4-Trichlorobenzene	10.0
91-20-3	Naphthalene	10.0
106-47-8	4-Chloroaniline	10.0
87-68-3	Hexachlorobutadiene	10.0
59-50-7	4-Chloro-3-methylphenol	10.0
91-57-6	2-Methylnaphthalene	10.0
77-47-4	Hexachlorocyclopentadiene	10.0
88-06-2	2,4,6-Trichlorophenol	10.0
95-95-4	2,4,5-Trichlorophenol	50.0
91-58-7	2-Chloronaphthalene	10.0
88-74-4	2-Nitroaniline	50.0
131-11-3	Dimethyl phthalate	10.0
208-96-8	Acenaphthylene	10.0
99-09-2	3-Nitroaniline	50.0
83-32-9	Acenaphthene	10.0
51-28-5	2,4-Dinitrophenol	50.0
100-02-7	4-Nitrophenol	50.0
132-64-9	Dibenzofuran	10.0
606-20-2	2,6-Dinitrotoluene	10.0
121-14-2	2,4-Dinitrotoluene	10.0
84-66-2	Diethylphthalate	10.0
7005-72-3	4-Chlorophenyl-phenylether	10.0
86-73-7	Fluorene	10.0
100-01-6	4-Nitroaniline	50.0

TABLE 3. (CONTINUED) - SEMI-VOLATILE ORGANICS CON'T.

C.A.S. Number	Compound Name	Quantitation Limits (ug/L)
86-30-6	N-Nitrosodiphenylamine	10.0
103-33-3	1,2-Diphenylhydrazine	10.0
101-55-3	4-Bromophenyl-phenylether	10.0
87-86-5	Pentachlorophenol	50.0
85-01-8	Phenanthrene	10.0
120-12-7	Anthracene	10.0
84-74-2	Di-n-Butylphthalate	10.0
118-74-1	Hexachlorobenzene	10.0
206-44-0	Fluoranthene	10.0
92-87-5	Benzidine	50.0
129-00-0	Pyrene	10.0
85-68-7	Butylbenzylphthalate	10.0
91-94-1	3,3'-Dichlorobenzidine	20.0
56-55-3	Benzo(a)anthracene	10.0
117-81-7	Bis(2-ethylhexyl)phthalate	10.0
218-01-9	Chrysene	10.0
117-84-0	Di-n-octyl phthalate	10.0
205-99-2	Benzo(b)fluoranthene	10.0
207-08-9	Benzo(k)fluoranthene	10.0
50-32-8	Benzo(a)pyrene	10.0
193-39-5	Indeno(1,2,3-cd)pyrene	10.0
53-70-3	Dibenzo(a,h)anthracene	10.0
191-24-2	Benzo(g,h,i)perylene	10.0

TABLE 3. (CONTINUED) - PESTICIDES/PCBs AND HERBICIDES.

C.A.S. Number	Parameter	Quantitation Limits (ug/L)
	Pesticides/PCBs	
319-84-6	Alpha-BHC	0.02
319-87-7	Beta-BHC	0.02
319-86-8	Delta-BHC	0.02
58-89-9	Lindane	0.02
76-44-8	Heptachlor	0.02
309-00-2	Aldrin	0.02
1024-57-3	Heptachlor epoxide	0.02
959-98-8	Endosulfan I	0.02
60-57-1	Dieldrin	0.02
75-55-9	4,4'-DDE	0.02
72-20-8	Endrin	0.02
33213-65-9	Endosulfan II	0.02
72-54-8	4,4'-DDD	0.02
1031-07-8	Endosulfan sulfate	0.02
50-29-3	4,4'-DDT	0.02
72-43-5	Methoxychlor	0.02[v]
7421-93-4	Endrin aldehyde	0.02[w]
53494-70-5	Endrin ketone	0.02[x]
57-74-9	Chlordane	0.16[w,y]
5103-71-9	Alpha chlordane	0.16[x]
5103-74-2	Gamma chlordane	0.16[x]
8001-35-2	Toxaphene	1.0
12674-11-2	Aroclor-1016	0.20
11104-28-2	Aroclor-1221	0.20
11141-16-5	Aroclor-1232	0.20
53469-21-9	Aroclor-1242	0.20
12672-29-6	Aroclor-1248	0.20
11097-69-1	Aroclor-1254	0.20
11096-82-5	Aroclor-1260	0.20
	Herbicides	
94-75-7	2,4-D	0.1[z]
93-72-1	Silvex	0.1[z]
93-76-5	2,4,5-T	0.1[z]

[a] Quantitation limit was 1.0 mg/L during the 07/24/89 analysis.
[b] Quantitation limit was 10.0 mg/L during the 11/16/89 and 12/07/89 analyses.
[c] Quantitation limit was 0.04 mg/L during the 09/07/89 analysis.
[d] Quantitation limit was 0.1 mg/L during the 07/24/89 analysis
[e] Quantitation limit was 0.01 mg/L during the 09/07/89 analysis.

TABLE 3. (CONTINUED) - FOOTNOTES CON'T.

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[f] Quantitation limit was 1.0 mg/L during the 07/24/89 and 09/07/89 analyses.
[g] Quantitation limit was 0.5 mg/L during the 09/07/89 analysis.
[h] Quantitation limit was 0.1 mg/L during the 07/24/89 and 0.5 mg/L during the 11/16/89 analyses.
[i] Quantitation limit was 0.1 mg/L during the 11/16/89 and 12/07/89 analyses.
[j] Quantitation limit was 0.2 mg/L during the 12/07/89 analysis.
[k] Quantitation limit was 5.0 mg/L during the 07/24/89 and 12/07/89 analyses.
[l] Quantitation limit was 0.03 mg/L during the 09/07/89 analysis.
[m] Quantitation limit was 0.05 mg/L during the 11/16/89 and and 0.01 mg/L 12/07/89 analyses.
[n] Quantitation limit was 2.0 mg/L during the 07/24/89 and 5.0 mg/L 12/07/89 analyses.
[o] Quantitation limit was 0.015 mg/L during the 07/24/89 analysis.
[p] Quantitation limit was 0.02 mg/L during the 07/24/89 analysis.
[q] Quantitation limit was 1.0 mg/L during the 11/16/89 and 12/07/89 analyses.
[r] Volatile organics not measured during the 11/16/89 analysis.
[s] Compound measured during the 09/28/89 and 10/26/89 analyses.
[t] Compound not measured during the 09/28/89 analysis.
[u] Compound measured during the 7/24/89, 9/28/89, and 10/26/89 analyses.
[v] Compound not analyzed during the 06/21/89 and 07/24/89 analyses.
[w] Compound not analyzed during the 12/07/89 analysis.
[x] Compound analyzed during the 12/07/89 analysis.
[y] Practical quantitation limit was 0.02 mg/L during the 09/07/89 and 09/28/89 analyses.
[z] Effluent sample not analyzed for herbicides because of a sample identity problem during the 11/16/89 analysis at Biospherics Inc..

TABLE 4. SUMMARY OF THE ANALYTICAL METHODS USED FOR THE APG-EA WWTP EFFLUENT, APG DILUENT WATER AND JHU/APL-AES DILUENT WATER CHEMICAL ANALYSES.

Parameter	Method	Reference
Metals	EPA 3010/3020, EPA 6010/7000s,	(USEPA, 1986)
	EPA 200.0/200.7/245.1[a]	(USEPA, 1983)
Mercury	EPA 7470	(USEPA, 1986)
	EPA 245.1[b]	(USEPA, 1983)
Alkalinity	EPA 310.2/310.1[c]	(USEPA, 1983)
Ammonia	EPA 350.1	(USEPA, 1983)
Cyanide	EPA 335.2	(USEPA, 1983)
Phosphorous	EPA 365.3/365.2[d]	(USEPA, 1983)
Sulfide	EPA 376.1	(USEPA, 1983)
Nitrate/Nitrite	EPA 353.2	(USEPA, 1983)
Nitrite	EPA 354.1	(USEPA, 1983)
Nitrate	EPA 300.0	(USEPA, 1983)
Conductivity	EPA 120.1	(USEPA, 1983)
Total suspended solids	EPA 160.2/160.1[e]	(USEPA, 1983)
Fluoride	EPA 340.2	(USEPA, 1983)
Sulfate	EPA 375.4/300.1[f]	(USEPA, 1983)
Chloride	EPA 325.3/300.1/300.0[g]	(USEPA, 1983)
Hardness	EPA 130.2	(USEPA, 1983)
	SM 314 A[h]	(APHA et al., 1985)
Volatile organics	EPA 8240	(USEPA, 1986)
	EPA 624[i]	(USEPA, 1983)
Semi-volatile organics	EPA 8270	(USEPA, 1986)
	EPA 625[j]	(USEPA, 1983)

TABLE 4. (CONTINUED).

Parameter	Method	Reference
Herbicides	EPA 615	(USEPA, 1983)
Pesticide/PCB's	EPA 8080	(USEPA, 1986)

[a] EPA 200.0/200.7/245.1 used during the 12/07/89 analysis.
[b] EPA 7470 used during the 09/07/89 analysis.
[c] EPA 310.2 used during the 09/07/89 analysis.
[d] EPA 365.3 used during the 09/07/89 analysis.
[e] EPA 160.1 used during the 12/07/89 analysis.
[f] EPA 300.1 used during the 10/26/89 analysis.
[g] EPA 300.1 and 300.0 used during the 10/26/89 and 12/07/89 analyses.
[h] Standard Method 314 A used during the 10/26/89 analysis.
[i] EPA 8240 used during the 09/07/89 analysis.
[j] EPA 8270 used during the 09/07/89 analysis.

TABLE 5. ROUTINE WATER QUALITY ANALYSES AND METHODS OF ANALYSIS FOR ALL GRAB SAMPLES TAKEN IN THE BIOMONITORING TRAILER AND ALL SAMPLES ANALYZED AT THE JHU/APL-AES LABORATORY.

Parameter	Method[a]
Alkalinity	Method 403
Conductivity	Method 205
Dissolved Oxygen	Method 421 F. Membrane Electrode Method
Hardness	Method 314 B. EDTA Titrimetric Method
pH	Method 423
Temperature	Method 212

[a] All methods taken from Standard Methods (APHA et al., 1985).

TABLE 6. MICROTOX® TEST RESULTS ON 24-HOUR COMPOSITE SAMPLES OF APG-EA WWTP EFFLUENT.

Date of Sample	Microtox® 15-min EC50[a]
June 05	-
06	-
13	-
14	-
15	-
16	-
17	-
18	-
19	-
20	-
21	-
22	-
23	-
25	-
26	-
27	-
28	-
29	-
30	-
July 01	-
02	-
03	-
04	-
05	-
06	-
07	-
08	-
09	-
10	-
11	-
12	-
13	-
14	-
15	-
16	-
17	-
18	-
19	-
20	18.8
21	-
22	-
23	-
24	1.1

TABLE 6. (CONTINUED).

Date of Sample		Microtox® 15-min EC50[a]
July	25	-
	26	3.1
	27	-
	28	-
	29	-
	30	-
	31	-
Aug	01	-
	02	-
	03	-
	04	-
	06	b
	07	b
	08	b
	09	-
	10	-
	11	-
	12	c
Oct	06	-
	07	-
	08	-
	09	-
	10	-
	11	-
	12	-
	13	-
	14	-
	15	-
	16	-
	17	-
	18	-
	19	-
	20	-
	21	-
	22	-
	23	-
	24	-
	25	-
	26	-
	27	-
	28	-
	29	-
	30	-
	31	-

TABLE 6. (CONTINUED).

Date of Sample		Microtox® 15-min EC50[a]
Nov	01	-
	02	-
	03	-
	04	-
	05	-
	06	-
	07	-
	16	-
	20	-
	27	-
Dec	07	-
	12	-

[a] EC50s are expressed as percent effluent by volume.
[b] EC50s were shown by the Microtox® instrument to be >100% effluent by volume; thus, the EC50s were rejected.
[c] Instrument was sent to Microbics Corp. for repair; instrument was returned October 3.

TABLE 7. SUMMARY OF THE AMES MUTAGENICITY ASSAY RESULTS.

Date of Sample	Parameter	Result
07/24/89	Effluent- unconcentrated	No mutagenic activity
	Effluent- concentrated (10X)	Mutagenic activity[a]
09/07/89	Effluent- unconcentrated	No mutagenic activity
	Effluent- concentrated (10X)	Mutagenic activity[a]
09/28/90	Effluent- unconcentrated	No mutagenic activity
	Effluent- concentrated (10X)	Mutagenic activity[a]
10/26/89	Effluent- unconcentrated	No mutagenic activity
	Effluent- concentrated (10X)	Mutagenic activity[a]
11/16/89	Effluent- unconcentrated	No mutagenic activity
	Effluent- concentrated (10X)	Mutagenic activity[a]
	Diluent Water- unconcentrated	No mutagenic activity
	Diluent Water- concentrated (10X)	No mutagenic activity

TABLE 7. (CONTINUED).

Date of Sample	Parameter	Result
12/07/89	Effluent- unconcentrated	No mutagenic activity
	Effluent- concentrated (10X)	Mutagenic activity[a]
	Diluent Water- unconcentrated	No mutagenic activity
	Diluent Water- concentrated (10X)	No mutagenic activity

[a] Refer to Section 4.3 for further information.

TABLE 8. RESULTS OF THE APG-EA WWTP EFFLUENT AND APG DILUENT WATER COMPREHENSIVE CHEMICAL ANALYSES - EFFLUENT GENERAL WATER QUALITY.[a]

Parameter	Date of Sample			
	06/21/89	07/24/89	09/07/89	09/28/89
Alkalinity	32	44	40	b
Ammonia-nitrogen	0.11	<0.04	0.05	1.53
Cyanide	<0.01	<0.01	<0.01	<0.01
Hardness	86	90	94	104
Nitrite	<0.04	b	0.05	b
Nitrate	5.07	b	6.45	b
Nitrate/nitrite combined as N	b	3.5	b	8.72
Phosphorous	0.73	0.55	1.19	0.85
Sulfide	7.4	1.8	<2	<2
Conductivity (umho/cm)	371	314	599	473
Total suspended solids	8	<1	<1	8
Fluoride	0.68	0.70	0.84	b
Sulfate	25	33	31	27
Chloride	57	59	127	85

TABLE 8. (CONTINUED) - EFFLUENT GENERAL WATER QUALITY CON'T.[a]

Parameter	Date of Sample		
	10/26/89	11/16/89	12/07/89
Alkalinity	44	63	34
Ammonia-nitrogen	0.54	0.66	0.35
Cyanide	<0.01	0.01	<0.01
Hardness	90	95	45
Nitrite	[b]	0.16	<0.02
Nitrate	[b]	9.26	4.4
Nitrate/nitrite combined as N	5.2	[b]	[b]
Phosphorous	0.93	0.70	0.60
Sulfide	<2	13	<2
Conductivity (umho/cm)	324	[c]	437
Total suspended solids	<5	[c]	6
Fluoride	0.62	[c]	0.67
Sulfate	44	[c]	40.8
Chloride	56	[c]	65

TABLE 8. (CONTINUED) - DILUENT WATER GENERAL WATER QUALITY CON'T.[a]

Parameter	Date of Sample			
	06/21/89	07/24/89	09/07/89	09/28/89
Alkalinity	32	<1	36	[b]
Ammonia-nitrogen	<0.04	0.04	0.33	<0.04
Cyanide	<0.01	<0.01	<0.01	<0.01
Hardness	71	68	30	66
Nitrite	<0.04	[b]	<0.04	[b]
Nitrate	2.10	[b]	<0.04	[b]
Nitrate/nitrite combined as N	[b]	1.9	[b]	2.10
Phosphorous	0.09	0.04	0.16	0.07
Sulfide	2.9	1.0	<2	<2
Conductivity (umho/cm)	191	745	180	176
Total suspended solids	2	<1	<1	<5
Fluoride	1.00	0.87	1.04	[b]
Sulfate	16	38	12	17.2
Chloride	16	13	10	18

TABLE 8. (CONTINUED) - DILUENT WATER GENERAL WATER QUALITY CON'T.[a]

Parameter	Date of Sample		
	10/26/89	11/16/89	12/07/89
Alkalinity	34	31	30
Ammonia-nitrogen	0.1	<0.04	0.06
Cyanide	<0.01	0.01	<0.01
Hardness	67	71	31
Nitrite	[b]	<0.02	<0.02
Nitrate	[b]	6.21	1.7
Nitrate/nitrite combined as N	2.0	[b]	[b]
Phosphorous	0.08	0.06	0.05
Sulfide	<2	10	<2
Conductivity (umho/cm)	160	[c]	148
Total suspended solids	<5	[c]	<5
Fluoride	0.79	[c]	0.70
Sulfate	17.9	[c]	10.8
Chloride	20.9	[c]	11

TABLE 8. (CONTINUED) - METALS (MG/L) IN EFFLUENT AND DILUENT WATER.[d]

Parameter	Date of Sample						
	06/21/89	07/24/89	09/07/89	09/28/89	10/26/89	11/16/89	12/07/89
				Effluent			
Aluminum		0.30					
Boron	0.11	0.12	0.10	0.066		0.11	0.04
Calcium	22.6	21.2	25.4	28.3	23.8	25.7	18.0
Copper						0.024	
Iron	0.52	0.46	1.01	0.52	0.42	0.59	0.64
Magnesium	6.72	6.46	7.55	8.56	7.51	7.46	
Manganese	0.06	0.043	0.05	0.037	0.035	0.047	
Potassium	4.00	4.04	5.67	6.80	5.73	15.1	2.2
Sodium	31.3	34.2	80.2	52.6	35.9	35.4	40.1
				Diluent Water			
Aluminum		0.24			0.2		
Boron	0.068					0.062	
Calcium	16.8	14.0	17.3	19.3	18.7	19.3	12.0
Copper						0.023	
Iron							0.11
Magnesium	4.17	3.72	4.85	5.07	4.91	5.43	
Manganese		0.023					
Potassium	1.83	2.16	2.02	2.70	2.96	5.9	
Sodium	4.93	6.14	9.53	7.37	7.41	9.5	7.9

TABLE 8. (CONTINUED) - VOLATILE AND SEMI-VOLATILE ORGANICS (MICROGRAMS/L) IN EFFLUENT AND DILUENT WATER.[d]

Parameter	Date of Sample						
	06/21/89	07/24/89	09/07/89	09/28/89	10/26/89	11/16/89	12/07/89
				Effluent			
Chloroform	7.0	9.0		10.0	8.0		
				Diluent Water			
Chloroform	11.0	21.0		6.0	12.0		
1,1,1-Trichloroethane							5.0
Di-n-octyl phthalate							10.0

[a] Concentration is mg/L for all parameters except conductivity which is umho/cm.
[b] Analysis not conducted.
[c] Analysis not conducted due to confusion by vendor over sample identity.
[d] Only compounds detected at or above the quantitation limits in Table 3 are reported.

TABLE 9. RAW AND MEAN WATER QUALITY DATA FROM ONE EFFLUENT AND ONE DILUENT WATER TREATMENT TANK IN CARCINOGENICITY TEST (O) - 100% EFFLUENT, 20 MG/L DEN EXPOSURE (TANK NO. 3).

Date	Temperature (°C)	pH	Dissolved Oxygen (mg/L)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
06/18/89	24.6	6.85	7.6	48	120
06/25/89	24.9	6.95	7.7	48	103
07/03/89	25.0	7.00	7.7	48	120
07/10/89	25.0	6.80	7.8	48	120
07/18/89	25.1	6.65	7.7	54	120
07/26/89	25.3	6.60	7.6	48	103
07/31/89	25.1	6.70	7.7	54	103
08/08/89	25.0	6.85	7.2	54	103
08/16/89	25.1	6.75	7.4	54	103
08/21/89	25.0	6.90	7.3	48	103
09/04/89	25.1	6.70	7.2	48	120
09/12/89	25.1	6.80	7.0	48	120
09/19/89	25.0	6.75	7.3	48	120
09/26/89	25.3	6.70	6.9	48	120
10/02/89	25.7	6.80	6.5	54	103
10/09/89	23.8	6.70	7.4	48	103
10/18/89	24.8	6.80	6.6	61	86
10/23/89	24.0	6.70	6.8	54	103
10/30/89	24.0	6.70	7.0	54	120
11/06/89	24.2	6.80	7.8	48	103
11/14/89	25.2	6.70	6.6	48	103
11/20/89	25.6	7.00	6.8	48	120
11/27/89	25.7	6.93	7.7	48	103
12/04/89	22.3	6.82	8.1	41	86
12/11/89	24.3	7.25	7.7	61	103

TABLE 9. (CONTINUED) - 100% EFFLUENT, 20 MG/L DEN EXPOSURE (TANK NO. 3) CON'T.

Statistical Parameters	Temperature (°C)	pH	Dissolved Oxygen (mg/L)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
Mean	24.8	6.81	7.3	50	108
Std Dev	0.72	0.138	0.44	4.7	10.5
Min Value	22.3	6.60	6.5	41	86
Max Value	25.7	7.25	8.1	61	120
N	25	25	25	25	25

TABLE 9. (CONTINUED) - DILUENT WATER, 20 MG/L DEN EXPOSURE (TANK NO. 6).

Date	Temperature (°C)	pH	Dissolved Oxygen (mg/L)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
06/18/89	24.6	7.00	8.2	41	86
06/25/89	24.8	6.95	7.7	41	86
07/03/89	24.8	6.95	8.1	41	86
07/10/89	25.0	6.85	8.0	41	86
07/18/89	25.0	6.65	7.8	41	86
07/26/89	25.1	6.65	7.9	41	86
07/31/89	25.0	6.60	7.8	41	86
08/08/89	24.9	6.70	7.6	41	86
08/16/89	25.0	6.65	7.7	41	86
08/21/89	25.1	7.65	7.5	41	86
09/04/89	25.0	6.80	7.4	41	86
09/12/89	25.0	6.70	7.2	41	86
09/19/89	25.0	7.00	7.5	41	86
09/26/90	25.3	6.85	7.7	41	86
10/02/89	25.5	6.90	7.3	48	86
10/09/89	24.0	6.95	7.8	41	68
10/18/89	24.7	6.95	7.5	41	86
10/23/89	24.0	6.80	7.7	41	68
10/30/89	24.2	6.70	7.6	48	86
11/06/89	24.2	6.75	8.2	41	86
11/14/89	25.1	6.70	7.8	41	86
11/20/89	25.2	7.03	7.9	41	86
11/27/89	25.4	6.75	8.0	41	86
12/04/89	22.9	6.99	8.0	41	86
12/11/89	24.0	7.11	7.6	41	86

TABLE 9. (CONTINUED) - DILUENT WATER, 20 MG/L DEN EXPOSURE (TANK NO. 6) CON'T.

Statistical Parameters	Temperature (°C)	pH	Dissolved Oxygen (mg/L)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
Mean	24.8	6.87	7.7	41	84
Std Dev	0.57	0.213	0.26	1.8	4.6
Min Value	22.9	6.60	7.2	41	68
Max Value	25.5	7.65	8.2	48	86
N	25	25	25	25	25

APPENDIX 1

ROTIFER 24-H ACUTE TEST

Test Method:	Rotifer ToxKit™ Screening Test (US TOXKIT, Tampa, FL)
Type of Test:	Static
Date:	June 21-23, 1989
Investigator:	G. T. Peters
Laboratory:	JHU/APL-AES
Effluent:	
Source:	APG-EA WWTP
Chemical Characteristics:	Effluent not analyzed during test; however, see Tables 3 and 8 in text
Test Medium:	Rotifer ToxKit™ synthetic medium
Test Organism:	
Scientific Name:	*Brachionus rubens*
Wet Weight:	n/a
Length:	n/a
Age:	<4 h after hatch
Source:	Rotifer ToxKit™ cyst
Experimental Chambers:	
Material:	Glass Petri dish
Volume:	10 mL
No. Organisms Per Treatment:	10
Loading:	n/a
Lighting:	Fluorescent; 60-85 foot candles
Metering System:	n/a
Flow Rate:	n/a
Aeration:	No aeration during test

Endpoint:	Mortality
General Chemistry of Medium:	
Dissolved Oxygen:	7.7 mg/L APHA Standard Methods (1985)
pH:	7.4-7.7 APHA Standard Methods (1985)
Alkalinity:	60-70 mg/L as $CaCO_3$ APHA Standard Methods (1985)
Hardness:	80-100 mg/L as $CaCO_3$ APHA Standard Methods (1985)
Temperature:	25 ± 0.5°C

Results: The effluent did not affect survival. The data are summarized in Table A1-1.

TABLE A1-1. SURVIVAL OF ROTIFERS AFTER 24 HOURS EXPOSURE TO APG-EA WWTP EFFLUENT.

Parameter	Rep	Number Tested	No. Alive at End of Test	Percent Alive
Growth medium	A	10	9	90
	B	10	8	80
	C	10	9	90
100% Effluent	A	9	8	89
	B	9	9	100
	C	8	7	88

APPENDIX 2

ROTIFER 24-H ACUTE TEST

Test Method:	Rotifer ToxKit™ Screening Test (US TOXKIT, Tampa, FL)
Type of Test:	Static
Date:	October 31 - November 2, 1989
Investigator:	S. D. Turley
Laboratory:	JHU/APL-AES
Effluent:	
Source:	APG-EA WWTP
Chemical Characteristics:	Effluent not analyzed during test; however, see Tables 3 and 8 in text
Test Medium:	Rotifer ToxKit™ synthetic medium
Test Organism:	
Scientific Name:	*Brachionus rubens*
Wet Weight:	n/a
Length:	n/a
Age:	<4 h after hatch
Source:	Rotifer ToxKit™ cyst
Experimental Chambers:	
Material:	Glass Petri dish
Volume:	10 mL
No. Organisms Per Treatment:	10
Loading:	n/a
Lighting:	Fluorescent; 60-85 foot candles
Metering System:	n/a
Flow Rate:	n/a
Aeration:	No aeration during test

Endpoint:	Mortality
General Chemistry of Medium:	
Dissolved Oxygen:	7.5 mg/L (Range 7.0-8.0) APHA Standard Methods (1985)
pH:	7.8 (Range 7.4-8.0) APHA Standard Methods (1985)
Conductivity:	383 umhos/cm (Range 330-435) APHA Standard Methods (1985)
Alkalinity:	80 mg/L as $CaCO_3$ (Range 50-110) APHA Standard Methods (1985)
Hardness:	156 mg/L as $CaCO_3$ (Range 92-220) APHA Standard Methods (1985)
Temperature:	25 ± 0.5°C

Results: The effluent did not affect survival. The data are summarized in Table A2-1.

TABLE A2-1. SURVIVAL OF ROTIFERS AFTER 24 HOURS EXPOSURE TO APG-EA WWTP EFFLUENT.

Concentration (% Effluent by Volume)	Rep	Number Tested	No. Alive at End of Test	Percent Alive
Growth Medium	A	10	10	100
	B	10	10	100
	C	10	9	90
6.25	A	10	9	90
	B	10	10	100
	C	10	8	80
12.5	A	10	9	90
	B	10	9	90
	C	10	10	100
25	A	10	8	80
	B	10	8	80
	C	10	9	90
50	A	10	7	70
	B	10	10	100
	C	10	8	80
100	A	10	9	90
	B	10	7	70
	C	10	9	90

Results: The effluent did not affect the survival of the organisms. The statistical analysis of the data is summarized on the next page.

Statistical Analysis of Rotifer Survival

Data Transformation:

Arc-sine square-root transformation

Chi-Square Test for Normality:

Calculated test statistic:	11.97
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	3.04
Alpha value:	0.01
Critical value:	15.09
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	1.11
Alpha value:	0.05
Critical value:	3.11
Conclusion:	Fail to reject the null hypothesis that all groups are equal

APPENDIX 3

GREEN ALGAL 96-H GROWTH TEST

Test Method:	Horning and Weber (1985)
Type of Test:	Static
Date:	July 25-29, 1989
Investigators:	G. T. Peters S. D. Turley
Laboratory:	JHU/APL-AES
Effluent:	
Source:	APG-EA WWTP
Chemical Characteristics:	Effluent not analyzed during test; however, see Tables 3 and 8 in text
Test Medium:	Double strength "AAP" medium (Miller et al., 1978) with P added to achieve a 20:1 N:P atomic ratio.
Test Organism:	
Scientific Name:	Selenastrum capricornutum
Age:	Log growth
Source:	University of Texas culture collection
Experimental Chambers:	
Material:	Glass culture flasks; stoppered with cheesecloth/cotton
Volume:	500 mL
Initial Cell Density:	5×10^3 cells/mL
Lighting:	Fluorescent; cool white; continuous; ≈300 foot candles
Aeration:	None
Endpoint:	Reduction in growth rate relative to control

Temperature: 20 ± 0.5°C

Results: The effluent did not affect growth rate. The data are summarized in Tables A3-1 and A3-2.

TABLE A3-1. MEAN CELL DENSITY (CELLS/ML) OF GREEN ALGA EXPOSED TO APG-EA WWTP EFFLUENT.

Conc (Percent Effluent by Vol)	Rep	Mean Cell Density				
		0H	24H	48H	72H	96H
Growth Medium	1	7608	31360	333520	901120	1019453
	2	4688	18157	279640	782133	981320
	3	5435	29272	232493	821533	959147
6.25%	1	5480	35789	180947	1042506	1176653
	2	9944	36261	368960	892600	1215866
	3	8275	29900	332933	803360	991293
12.5	1	4005	38704	166053	839960	896267
	2	3477	44298	232880	830493	1165826
	3	3893	61056	240600	880293	1407933
25.0	1	4098	47189	206080	1034866	1373106
	2	6451	69053	196666	1041133	1445386
	3	4549	48648	170213	1284000	1422720
50.0	1	4688	56209	260720	1138533	1514040
	2	7221	64416	88920	1187346	1365666
	3	10208	67091	107000	1012506	1634626
99.0	1	6106	38507	78880	971120	1463626
	2	4187	52941	105640	915413	1196146
	3	3893	51341	46640	804373	1094547

TABLE A3-2. GROWTH RATE OF GREEN ALGA AFTER 96 HOURS OF EXPOSURE TO APG-EA WWTP EFFLUENT.

Concentration (Percent Effluent by Vol)	Rep	Growth Rate Per Day[a]	Mean Growth Rate Per Day	Relative Growth Rate
Growth Medium	1	0.5318		
	2	0.5803		
	3	0.5617	0.5580	100.0
6.25	1	0.5831		
	2	0.5218		
	3	0.5196	0.5415	97.1
12.5	1	0.5875		
	2	0.6314		
	3	0.6398	0.6195	110.0
25.0	1	0.6319		
	2	0.5876		
	3	0.6238	0.6145	110.1
50.0	1	0.6273		
	2	0.5693		
	3	0.5511	0.5826	104.4
99.0	1	0.5949		
	2	0.6140		
	3	0.6127	0.6072	108.8

[a] Growth Rate = $\log_{10}n_1 - \log_{10}n_2 / t_1 - t_2$, where

n_1 = cell density (cells/mL) at day 4

n_2 = cell density (cells/mL) at day 0

t = time in days.

APPENDIX 4

GREEN ALGAL 96-H GROWTH TEST

Test Method:	Horning and Weber (1985)
Type of Test:	Static
Date:	October 29 - November 2, 1989
Investigator:	S. D. Turley
Laboratory:	JHU/APL-AES
Effluent:	
Source:	APG-EA WWTP
Chemical Characteristics:	Effluent not analyzed during test; however, see Tables 3 and 8 in text
Test Medium:	Double strength "AAP" medium (Miller et al., 1978) with P added to achieve a 20:1 N:P atomic ratio.
Test Organism:	
Scientific Name:	*Selenastrum capricornutum*
Age:	Log growth
Source:	University of Texas culture collection
Experimental Chambers:	
Material:	Glass culture flasks with cheesecloth/cotton stoppers
Volume:	500 mL
Initial Cell Density:	5×10^3 cells/mL
Lighting:	Fluorescent; cool white; continuous; ≈300 foot candles
Aeration:	None
Endpoint:	Reduction in growth rate relative to control
Temperature:	20 ± 0.5 °C

Results: The effluent did not affect growth rate. The data are summarized in Tables A4-1 and A4-2.

TABLE A4-1. MEAN CELL DENSITY (CELLS/ML) OF GREEN ALGA EXPOSED TO APG-EA WWTP EFFLUENT.

Conc (Percent Effluent by Vol)	Rep	Mean Cell Density				
		0H	24H	48H	72H	96H
Growth Medium	1	5893	23995	228400	713080	983747
	2	5557	21133	217800	740853	1047760
	3	5088	24810	238880	730733	945427
6.25	1	5312	25403	243253	740853	1055053
	2	5621	26899	247307	716320	1067613
	3	6443	24003	239347	727680	957507
12.5	1	4835	29723	258080	754320	1127213
	2	5016	28605	243347	796920	996173
	3	5469	29893	269120	705640	1109573
25.0	1	5533	26928	265493	825320	1131320
	2	6165	31075	282293	890760	1067493
	3	5312	30728	246933	759347	1131267
50.0	1	4483	28003	279067	765267	1010867
	2	7011	31885	269813	886507	1075853
	3	6856	31659	255680	788253	1137920
99.0	1	5237	31163	247880	797027	1055187
	2	5136	31890	255093	824600	918413
	3	4869	31179	278280	870413	1142520

TABLE A4-2. GROWTH RATE OF GREEN ALGA AFTER 96 HOURS OF EXPOSURE TO APG-EA WWTP EFFLUENT.

Concentration (% Effluent by Volume)	Rep	Growth Rate Per Day[a]	Mean Growth Rate Per Day	Relative Growth Rate
Growth Medium	1	0.5558		
	2	0.5691		
	3	0.5675	0.5641	100.0
6.25	1	0.5747		
	2	0.5698		
	3	0.5432	0.5626	99.7
12.5	1	0.5920		
	2	0.5745		
	3	0.5771	0.5812	103.0
25.0	1	0.5774		
	2	0.5597		
	3	0.5821	0.5731	101.6
50.0	1	0.5883		
	2	0.5465		
	3	0.5550	0.5633	99.9
99.0	1	0.5761		
	2	0.5632		
	3	0.5926	0.5773	102.3

[a] Growth Rate = $\log_{10}n_1 - \log_{10}n_2 / t_1 - t_2$, where

n_1 = cell density (cells/mL) at day 4

n_2 = cell density (cells/mL) at day 0

t = time in days.

APPENDIX 5

CLADOCERAN 7-D SURVIVAL AND REPRODUCTION TEST

Test Method:	Waller and Lazorchak (1986)
Type of Test:	Static renewal (every 24 h)
Date:	June 15-22, 1989
Investigator:	G. T. Peters S. D. Turley
Laboratory:	JHU/APL-AES
Effluent:	
Source:	APG-EA WWTP
Chemical Characteristics:	Effluent not analyzed during test; however, see Tables 3 and 8 in text
Dilution Water:	
Source:	JHU/APL-AES deep well
Chemical Characteristics:	See Table 2 in text
Test Organism:	
Scientific Name:	*Ceriodaphnia dubia*
Wet Weight:	n/a
Length:	n/a
Age:	<6 h
Source:	JHU/APL-AES Culture
Experimental Chambers:	
Material:	50 mL glass beakers
Volume:	30 mL
No. Organisms Per Treatment:	10
Loading:	1 organism/beaker
Lighting:	Fluorescent; 60-85 foot candles
Metering System:	n/a
Flow Rate:	n/a

Aeration:	Prior to each renewal
Endpoints:	Mortality of adults; number of neonates produced in 3 broods

Mean Water Chemistry Values:

Dissolved Oxygen:	7.8 mg/L (Range 7.4-8.2) APHA Standard Methods (1985)
pH:	8.1 (Range 7.7-8.3) APHA Standard Methods (1985)
Conductivity:	325 umhos/cm (Range 295-365) APHA Standard Methods (1985)
Alkalinity:	97 mg/L as $CaCO_3$ (Range 35-130) APHA Standard Methods (1985)
Hardness:	159 mg/L as $CaCO_3$ (Range 104-196) APHA Standard Methods (1985)

Mean Temperatures:

Culture:	25°C (Range 24-26)
Test:	25°C (Range 24.5-25.5)

Results: The effluent did not affect the survival of the adults or the production of neonates. The data are summarized in Tables A5-1, A5-2, and A5-3.

TABLE A5-1. SURVIVAL OF DAPHNID ADULTS AFTER 7 DAYS OF EXPOSURE TO APG-EA WWTP EFFLUENT.

Concentration (% Effluent by Volume)	Number Tested	No. Alive at End of Test	Percent Alive
JHU/APL-AES Diluent Water	10	10	100
6.25	10	10	100
12.5	10	10	100
25.0	10	10	100
50.0	9	9	100
100	9	9	100

TABLE A5-2. SUMMARY OF LIVING DAPHNID OFFSPRING PRODUCED AFTER 7 DAYS OF EXPOSURE TO APG-EA WWTP.

Concentration (% Effluent by Volume)	N	Mean Number	Range
JHU/APL-AES Diluent Water	10	30.6	23 - 37
6.25	10	29.6	27 - 33
12.5	10	29.1	24 - 32
25.0	10	30.6	27 - 34
50.0	9	30.2	24 - 37
100	9	24.0	9 - 34

TABLE A5-3. NUMBER OF DAPHNID YOUNG PRODUCED PER BROOD, TOTAL NUMBER OF YOUNG, AND MEAN NUMBER OF YOUNG PER BROOD.

Concentration (% Effluent by Volume)	Rep	Brood No. 1	Brood No. 2	Brood No. 3	Total Young	Mean Young Per Brood
JHU/APL-AES Diluent Water	1	10	9	18	37	12.3
	2	4	15	16	35	11.7
	3	3	9	13	25	8.3
	4	3	8	12	23	7.7
	5	3	7	19	29	9.7
	6	5	11	18	34	11.3
	7	4	13	16	33	11.0
	8	4	10	17	31	10.3
	9	4	10	15	29	9.7
	10	4	10	16	30	10.0
6.25	1	4	9	16	29	9.7
	2	4	9	15	28	9.3
	3	5	9	16	30	10.0
	4	3	10	15	28	9.3
	5	6	10	14	30	10.0
	6	3	9	15	27	9.0
	7	4	11	18	33	11.0
	8	3	9	18	30	10.0
	9	3	9	16	28	9.3
	10	5	10	18	33	11.0
12.5	1	4	10	17	31	10.3
	2	5	10	15	30	10.0
	3	4	11	17	32	10.7
	4	4	7	13	24	8.0
	5	3	12	16	31	10.3
	6	5	11	14	30	10.0
	7	5	13	13	31	10.3
	8	4	10	15	29	9.7
	9	0	10	19	29	9.7
	10	3	9	12	24	8.0

TABLE A5-3. (CONTINUED).

Concentration (% Effluent by Volume)	Rep	Brood No. 1	Brood No. 2	Brood No. 3	Total Young	Mean Young Per Brood
25	1	3	12	19	34	11.3
	2	3	11	14	28	9.3
	3	5	11	14	30	10.0
	4	6	10	15	31	10.3
	5	3	11	17	31	10.3
	6	4	12	16	32	10.7
	7	0	11	16	27	9.0
	8	6	10	17	33	11.0
	9	0	13	16	29	9.7
	10	6	12	13	31	10.3
50	1	6	12	16	34	11.3
	2	4	12	21	37	12.3
	3	3	11	18	32	10.7
	4	5	9	13	27	9.0
	5	6	11	16	33	11.0
	6	6	11	17	34	11.3
	7	3	10	13	26	8.7
	8	4	7	14	25	8.3
	9	3	5	16	24	8.0
100	1	0	12	16	28	9.3
	2	5	10	19	34	11.3
	3	0	0	9	9	3.0
	4	2	4	14	20	6.7
	5	0	7	8	15	5.0
	6	4	8	12	24	8.0
	7	4	11	13	28	9.3
	8	4	12	16	32	10.7
	9	3	8	15	26	8.7

Results: The effluent did not affect the total number of neonates produced. The statistical analysis of the data is summarized on the next page.

Statistical Analysis of Total Daphnid Neonates Produced Per Adult

Data Transformation:

None

Chi-Square Test for Normality:

Calculate test statistic:	2.16
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartletts Test for Homogeneity of Variances:

Calculated test statistic:	24.62
Alpha value:	0.01
Critical value:	15.09
Conclusion:	Reject the null hypothesis that the variances are homogeneous

Kruskal-Wallis ANOVA by Ranks:

Calculated test statistic:	3.54
Alpha value:	0.05
Critical value:	11.07
Conclusion:	Fail to reject the null hypothesis that all treatments are equal

APPENDIX 6

CLADOCERAN 7-D SURVIVAL AND REPRODUCTION TEST

Test Method:	Waller and Lazorchak (1986)
Type of Test:	Static renewal (every 24 h)
Date:	October 5-12, 1989
Investigators:	S. D. Turley C. S. Lundmark
Laboratory:	JHU/APL-AES
Effluent:	
Source:	APG-EA WWTP
Chemical Characteristics:	Effluent not analyzed during test; however, see Tables 3 and 8 in text
Dilution Water:	
Source:	JHU/APL-AES deep well
Chemical Characteristics:	See Table 2 in text
Test Organism:	
Scientific Name:	*Ceriodaphnia dubia*
Wet Weight:	n/a
Length:	n/a
Age:	<12 h
Source:	JHU/APL-AES Culture
Experimental Chambers:	
Material:	50 mL glass beakers
Volume:	30 mL
No. Organisms Per Treatment:	10
Loading:	1 organism/beaker
Lighting:	Fluorescent; 60-85 foot candles
Metering System:	n/a
Flow Rate:	n/a

Aeration:	Prior to each renewal
Endpoints:	Mortality of adults; number of neonates produced in 3 broods
Mean Water Chemistry Values:	
Dissolved Oxygen:	7.3 mg/L (Range 7.0-7.5) APHA Standard Methods (1985)
pH:	8.1 (Range 7.8-8.3) APHA Standard Methods (1985)
Conductivity:	408 umhos/cm (Range 370-430) APHA Standard Methods (1985)
Alkalinity:	129 mg/L as $CaCO_3$ (Range 90-145) APHA Standard Methods (1985)
Hardness:	1[illegible]5 mg/L as $CaCO_3$ (Range 148-208) APHA Standard Methods (1985)
Mean Temperatures:	25°C (Range 24.5-25.5)

Results: The effluent did not affect the survival of the adults. A significant (alpha = 0.05) increase in neonate production occurred at all effluent concentrations relative to the controls; no difference in production of neonates occurred between effluent concentrations. The data are summarized in Tables A6-1, A6-2, A6-3, and A6-4.

TABLE A6-1. SURVIVAL OF DAPHNID ADULTS AFTER 7 DAYS OF EXPOSURE TO APG-EA WWTP EFFLUENT.

Concentration (% Effluent by Volume)	Number Tested	No. Alive at End of Test	Percent Alive
JHU/APL-AES Diluent Water	10	10	100
6.25	10	10	100
12.5	10	9	90
25.0	10	10	100
50.0	10	10	100
100	10	10	100

Results: The effluent did not affect the survival of the adults.

TABLE A6-2. SUMMARY OF LIVING DAPHNID OFFSPRING PRODUCED AFTER 7 DAYS OF EXPOSURE TO APG-EA WWTP.

Concentration (% Effluent by Volume)	N	Mean Number	Range
JHU/APL-AES Diluent Water	10	25.5	19 - 32
6.25	10	34.6	29 - 39
12.5	9	35.4	29 - 39
25.0	10	33.8	26 - 37
50.0	10	33.5	17 - 39
100	10	36.7	31 - 40

TABLE A6-3. NUMBER OF YOUNG PRODUCED PER BROOD, TOTAL NUMBER OF YOUNG, AND MEAN NUMBER OF YOUNG PER BROOD.

Concentration (% Effluent by Volume)	Rep	Brood No. 1	Brood No. 2	Brood No. 3	Total Young	Mean Young Per Brood
JHU/APL-AES Diluent Water	1	6	13	13	32	10.7
	2	5	11	12	28	9.3
	3	4	9	9	22	7.3
	4	5	11	12	28	9.3
	5	4	12	12	28	9.3
	6	4	11	9	24	8.0
	7	6	9	8	23	7.7
	8	2	7	10	19	6.3
	9	4	14	9	27	9.0
	10	6	8	10	24	8.0
6.25	1	6	12	18	36	12.0
	2	6	11	20	37	12.3
	3	6	14	17	37	12.3
	4	6	14	16	36	12.0
	5	5	11	16	32	10.7
	6	6	13	17	36	12.0
	7	6	13	12	31	10.3
	8	4	11	14	29	9.7
	9	6	10	17	33	11.0
	10	6	14	19	39	13.0
12.5	1	5	11	16	32	10.7
	2	5	11	20	36	12.0
	3	6	13	18	37	12.3
	4	5	13	21	39	13.0
	5	6	12	18	36	12.0
	6	6	13	18	37	12.3
	7	4	10	15	29	9.7
	8	6	12	20	38	12.7
	9	5	12	18	35	11.7
	10	*				

TABLE A6-3. (CONTINUED).

Concentration (% Effluent by Volume)	Rep	Brood No. 1	Brood No. 2	Brood No. 3	Total Young	Mean Young Per Brood
25	1	4	9	18	31	10.3
	2	6	12	17	35	11.7
	3	7	12	18	37	12.3
	4	7	11	18	36	12.0
	5	6	11	17	34	11.3
	6	6	11	18	35	11.7
	7	6	13	16	35	11.7
	8	5	13	16	34	11.3
	9	4	9	13	26	8.7
	10	6	12	17	35	11.7
50	1	1	12	16	29	9.7
	2	5	13	19	37	12.3
	3	2	12	17	31	10.3
	4	6	14	17	37	12.3
	5	6	15	18	39	13.0
	6	7	12	15	34	11.3
	7	7	12	19	38	12.7
	8	6	14	18	38	12.7
	9	6	13	16	35	11.7
	10	4	13	[a]	17	5.7
100	1	4	11	16	31	10.3
	2	5	13	19	37	12.3
	3	6	11	21	38	12.7
	4	7	12	17	36	12.0
	5	6	16	18	40	13.3
	6	6	13	19	38	12.7
	7	5	16	17	38	12.7
	8	5	13	17	35	11.7
	9	6	13	20	39	13.0
	10	6	12	17	35	11.7

[a] Daphnid died prior to end of test.

Results: The effluent did affect the total number of neonates produced. The statistical analysis of the data is summarized on the next page.

Statistical Analysis of Total Daphnid Neonates Produced Per Adult

Data Transformation:

None

Chi-Square Test for Normality:

Calculate test statistic:	6.85
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	11.26
Alpha value:	0.01
Critical value:	15.09
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	9.86
Alpha value:	0.05
Critical value:	2.45
Conclusion:	Reject the null hypothesis that all groups are equal

T-Test with Bonferroni Adjustment:

Calculated test statistic:	See Table A6-4
Alpha value:	0.05
Critical value:	2.40
Conclusion:	Reject the null hypothesis that all groups are equal

TABLE A6-4. RESULTS OF THE T-TEST WITH BONFERRONI ADJUSTMENT FOR TOTAL NUMBER OF DAPHNID NEONATES PRODUCED IN THREE BROODS.

Concentration (% Effluent by Volume)	N	Mean Number	T Statistic	Significance
JHU/APL-AES Diluent Water	10	25.5		
6.25	10	34.6	5.10	*
12.5	9	35.4	5.43	*
25	10	33.8	4.65	*
50	10	33.5	4.48	*
100	10	36.7	6.28	*

* Significantly different from the control at alpha = 0.05 (Bonferroni's critical value = 2.40).

APPENDIX 7

FATHEAD MINNOW 7-D SURVIVAL AND GROWTH TEST

Test Method:	Weber et al. (1989)
Type of Test:	Static renewal (every 24 h)
Date:	June 14-21, 1989
Investigators:	G. T. Peters S. D. Turley
Laboratory:	JHU/APL-AES
Effluent:	
Source:	APG-EA WWTP
Chemical Characteristics:	Effluent not analyzed during test; however, see Tables 3 and 8 in text
Dilution Water:	
Source:	JHU/APL-AES deep well
Chemical Characteristics:	See Table 2 in text
Test Organism:	
Scientific Name:	*Pimephales promelas*
Wet Weight:	0.38 mg
Age:	<24 h
Source:	JHU/APL-AES culture
Experimental Chambers:	
Material:	Glass aquaria; silicon sealant
Volume:	6.4 L
No. Organisms Per Replicate:	10
No. Organisms Per Treatment:	40
Loading:	<0.5 g/L
Lighting:	Fluorescent; 60-85 foot candles
Metering System:	n/a
Flow Rate:	n/a

Aeration:	None
Endpoints:	Mortality, growth
Mean Water Chemistry Values:	
Dissolved Oxygen:	7.3 mg/L (Range 6.9-7.5) APHA Standard Methods (1985)
pH:	7.7 (Range 7.4-8.0) APHA Standard Methods (1985)
Conductivity:	305 umhos/cm (Range 290-320) APHA Standard Methods (1985)
Alkalinity:	74 mg/L as $CaCo_3$ (Range 60-80) APHA Standard Methods (1985)
Hardness:	127 mg/L as $CaCo_3$ (Range 116-140) APHA Standard Methods (1985)
Temperature:	21.9°C (Range 21-22.7)

Results: Test was rejected because >20% mortality occurred in the controls. Although the test was rejected, the data indicate that the effluent did not cause greater mortality in any treatment group (relative to the controls) up to 100% effluent. In fact, survival was greater in all treatment groups than the control groups.

APPENDIX 8

FATHEAD MINNOW 7-D SURVIVAL AND GROWTH TEST

Test Method:	Weber et al. (1989)
Type of Test:	Static renewal (every 24 h)
Date:	October 27 - November 3, 1989
Investigators:	S. D. Turley C. S. Lundmark
Laboratory:	JHU/APL-AES
Effluent:	
Source:	APG-EA WWTP
Chemical Characteristics:	Effluent not analyzed during test; however, see Tables 3 and 8 in text
Dilution Water:	
Source:	JHU/APL-AES deep well
Chemical Characteristics:	See Table 2 in text
Test Organism:	
Scientific Name:	<u>Pimephales promelas</u>
Wet Weight:	1.03 mg
Age:	<24 h
Source:	JHU/APL-AES culture
Experimental Chambers:	
Material:	600 mL glass beakers
Volume:	500 mL
No. Organisms Per Replicate:	10
No. Organisms Per Treatment:	40
Loading:	<0.5 g/L
Lighting:	Fluorescent; 60-85 foot candles
Metering System:	n/a
Flow Rate:	n/a

Aeration:	None
Endpoints:	Mortality, growth
Mean Water Chemistry Values:	
Dissolved Oxygen:	7.2 mg/L (Range 6.4-7.7) APHA Standard Methods (1985)
pH:	7.8 (Range 7.6-8.4) APHA Standard Methods (1985)
Conductivity:	420 umhos/cm (Range 370-520) APHA Standard Methods (1985)
Alkalinity:	96 mg/L as $CaCo_3$ (Range 40-145) APHA Standard Methods (1985)
Hardness:	152 mg/L as $CaCo_3$ (Range 96-208) APHA Standard Methods (1985)
Temperature:	25°C (Range 24-26)

Results: The effluent did not affect the survival of the larvae. The effluent did affect the growth of the larvae. Statistically significant (alpha = 0.05) mortality occurred only in the 50% effluent concentration. The data are summarized in Tables A8-1, A8-2, and A8-3.

TABLE A8-1. SURVIVAL OF FATHEAD MINNOW LARVAE AFTER 7 DAYS OF EXPOSURE TO APG-EA WWTP EFFLUENT.

Concentration (% Effluent by Volume)	Rep	Number Tested	No. Alive at End of Test	Percent Alive
JHU/APL-AES Diluent Water	A	10	6	60
	B	10	7	70
	C	10	8	80
	D	10	7	70
6.25	A	10	8	80
	B	10	10	100
	C	10	10	100
	D	10	8	80
12.5	A	10	7	70
	B	10	9	90
	C	10	8	80
	D	10	10	100
25	A	10	7	70
	B	10	9	90
	C	10	8	80
	D	10	9	90
50	A	10	10	100
	B	10	7	70
	C	10	8	80
	D	10	8	80
100	A	10	10	100
	B	10	7	70
	C	10	9	90
	D	10	9	90

Results: The effluent did not affect the survival of the larvae. The analysis of the data is summarized on the next page.

Statistical Analysis of Fathead Minnow Larval Survival

Data Transformation:

Arc-sine square-root transformation

Shapiro-Wilk's Test for Normality:

Calculate test statistic:	0.97
Alpha value:	0.01
Critical value:	0.92
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	1.77
Alpha value:	0.01
Critical value:	15.09
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	1.36
Alpha value:	0.05
Critical value:	2.77
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE A8-2. GROWTH OF FATHEAD MINNOW LARVAE AFTER 7 DAYS OF EXPOSURE TO APG-EA WWTP EFFLUENT.

Concentration (% Effluent by Volume)	Rep	Dry Weight (mg)	Mean Dry Weight (mg)
JHU/APL-AES Diluent Water	A	0.72	
	B	0.51	
	C	0.56	
	D	0.59	0.60
6.25	A	0.54	
	B	0.46	
	C	0.41	
	D	0.49	0.48
12.5	A	0.54	
	B	0.50	
	C	0.39	
	D	0.45	0.47
25	A	0.53	
	B	0.47	
	C	0.39	
	D	0.61	0.50
50	A	0.44	
	B	0.40	
	C	0.28	
	D	0.31	0.36
100	A	0.47	
	B	0.40	
	C	0.61	
	D	0.47	0.49

Results: The effluent did affect the growth of the larvae. Statistically significant (alpha value = 0.05) mortality occurred only in the 50% effluent concentration. The analysis of the data is summarized on the next page and in Table A8-3.

Statistical Analysis of Fathead Minnow Larval Growth

Data Transformation:

None

Shapiro-Wilks Test for Normality:

Calculate test statistic:	0.95
Alpha value:	0.01
Critical value:	0.92
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	1.10
Alpha value:	0.01
Critical value:	15.09
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	3.83
Alpha value:	0.05
Critical value:	2.77
Conclusion:	Reject the null hypothesis that all groups are equal

Dunnett's Test:

Calculated test statistic:	See Table A8-3
Alpha value:	0.05
Critical value:	2.76
Conclusion:	Reject the null hypothesis that all groups are equal

TABLE A8-3. RESULTS OF DUNNETT'S TEST ON MEAN GROWTH OF FATHEAD MINNOW LARVAE AFTER 7 DAYS.

Concentration (% Effluent by Volume)	N	Mean Dry Weight (mg)	T Statistic	Significance
JHU/APL-AES Diluent Water	4	0.60		
6.25	4	0.48	2.19	
12.5	4	0.47	2.28	
25	4	0.50	1.73	
50	4	0.36	4.34	[a]
100	4	0.49	1.96	

[a] Significantly different at alpha = 0.05 (Dunnett's critical value = 2.76).

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